National Research
Council Canada

Conseil national
de recherches Canada

NRC · CNRC

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

http://www.nrc.ca/cisti/journals

ISSN 0706-652X / CJFSDX 57(7) 1321–1538 (2000)

The *Canadian Journal of Fisheries and Aquatic Sciences*, previously published as *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, and *Journal of the Fisheries Research Board of Canada* 1938–1979, is issued monthly to form annual volumes of 12 numbers plus supplements.

Editorial policy: The Journal publishes original research articles, perspectives (syntheses, critiques, re-evaluations), discussions (comment and reply), and rapid communications. Manuscripts may concern cells, organisms, populations, communities, ecosystems, or processes that affect aquatic systems. They may cover a range of disciplines including biology and ecology of marine and freshwater organisms, limnology, oceanography, physiology, toxicology, genetics, economics, disease, and management. Preference will be given to manuscripts that emphasize understanding of observed phenomena and interpretation of experimental results. The Journal encourages papers that lead from clearly stated purpose or rationale and from testable hypotheses, concepts, or questions to identifiable conclusions or syntheses. Such papers may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. The Journal discourages papers that are essentially descriptive, except in emerging disciplines; have only site-specific or local applications (a certain year, place, taxon, chemical compound, etc.); only confirm previously established principles; or apply standard techniques without breaking new methodological ground.

Manuscripts for publication should be submitted to the Editors, Canadian Journal of Fisheries and Aquatic Sciences, Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada (519-824-4120 ext. 2646; fax: 519-767-9798; e-mail: cjfas@uoguelph.ca).

Submissions in either English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. NRC accepts no responsibility for statements or opinions expressed by a contributor. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the *Journal*'s Editors or NRC. The use of proprietary names does not imply endorsement of the product or company.

Correspondence concerning accepted manuscripts and corrected proofs should be sent to NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Instructions to Authors are published once a year in the first issue of each volume. Copies are available from NRC Research Press or from our Web site. The **Publication Policy** of NRC Research Press is also available at our Web site:
http://www.nrc.ca/cisti/journals/rj.html

Microfilm: Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, U.S.A.

U.S. periodical postage paid at Champlain, N.Y., and at additional mailing offices. Attention U.S. POSTMASTER: Send address changes to **Canadian Journal of Fisheries and Aquatic Sciences**, c/o USACAN Media Distribution Center, P.O. Box 2888, Plattsburg, NY 12901, U.S.A.

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, et *Journal of the Fisheries Research Board of Canada* 1938–1979. Le *Journal* paraît tous les mois, formant ainsi un volume de 12 numéros, et offre en plus jusqu'à deux suppléments par année.

Politique éditoriale : On publie, dans le Journal, des articles rendant compte de recherche originale, des perspectives (synthèses, critiques et réévaluations), des discussions (commentaires et réponses) et des communications rapides. Les documents peuvent porter sur les cellules, organismes, populations, communautés, écosystèmes ou processus qui affectent les systèmes aquatiques. Ils peuvent relever de divers domaines y compris la biologie et l'écologie des organismes marins et d'eau douce, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, la pathologie et la gestion. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux. On invite les auteurs à soumettre des articles ayant des objectifs précis ou relevant d'hypothèses, de concepts ou de questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. On décourage toutefois la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicables à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; et d'articles rendant compte de l'application de normes sans gain de méthodologie.

Les auteurs voudront adresser leurs **manuscrits** aux directeurs scientifiques, Journal canadien des sciences halieutiques et aquatiques, Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada (519-824-4120 poste 2646; télécopieur : 519-767-9798; adresse électronique : cjfas@uoguelph.ca).

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à.-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le CNRC ne se tient en aucune façon responsable des opinions exprimées par un collaborateur ou des déclarations qui pourrait faire ce dernier. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par les directeurs du *Journal* ou du CNRC. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

La correspondance ayant trait aux manuscrits acceptés et les **épreuves corrigés** doivent être envoyées aux Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Recommandations aux auteurs sont publiés une fois par année dans le premier numéro de chaque volume. Des copies, ainsi que la **Politique de publication**, sont disponibles auprès des Presses scientifiques du CNRC ou au site Web :
http://www.nrc.ca/cisti/journals/rj.html

Microfilms : On peut acheter sur microfilms de 16 ou 35 mm tous les numéros depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, É.-U.

This journal is indexed or abstracted in / Ce journal est signalé ou résumé dans
ABSEARCH, Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Biological & Agricultural Index, CAB Abstracts, Chemical Abstracts, Current Contents, Elsevier BIOBASE, Environmental Abstracts, Environmental Periodicals Bibliography, GEOBASE, GeoRef, Life Sciences Collection, Meteorological and Geoastrophysical Abstracts, Oceanic Abstracts, PASCAL Database, and Science Citation Index.

Volume 57, Number 7, July 2000

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 57, numéro 7, juillet 2000

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

Editors / directeurs scientifiques John C. Roff and Moira M. Ferguson

Assistant to the Editors / adjointe aux directeurs scientifiques **Holly Foster**

Associate Editors / directeurs scientifique associés

D. Boisclair, *Université de Montréal*
U. Borgmann, *Environment Canada, Burlington*
S.E. Campana, *DFO/MPO, Dartmouth*
D.G. Dixon, *University of Waterloo*
A.E. Dizon, *National Marine Fisheries Service, La Jolla, Calif.*
J.J. Dodson, *Université Laval*
J.A. Downing, *Iowa State University*
K.T. Frank, *DFO/MPO, Dartmouth*
D.F. Houlihan, *University of Aberdeen, Aberdeen, Scotland*
F.A. Huntingford, *University of Glasgow*
J.A. Hutchings, *Dalhousie University*
G.S. Jamieson, *DFO/MPO, Nanaimo*
B. Jonsson, *Norwegian Institute for Nature Research, Oslo, Norway*
D.M. Mason, *NOAA-Great Lakes Environmental Research Laboratory, Ann Arbor, Mich.*
A. Mazumder, *University of Victoria*
D.L. Parrish, *Vermont Cooperative Fish and Wildlife Research Unit (USGS/BRD), University of Vermont*
P. Pepin, *DFO/MPO, St. John's*
E.E. Prepas, *University of Alberta*
T.M. Powell, *University of California, Berkeley, Calif.*
T.J. Quinn II, *University of Alaska–Fairbanks, Juneau, Alaska*
T.P. Quinn, *University of Washington*
R.D. Robarts, *Environment Canada, Saskatoon*
B. Sainte-Marie, *DFO/MPO, Mont-Joli*
D.W. Schindler, *University of Alberta*
S.J. Smith, *DFO/MPO, Dartmouth*
W.G. Sprules, *University of Toronto*
R.J. Thompson, *Memorial University of Newfoundland*
C.J. Walters, *University of British Columbia*
R.D. Ward, *CSIRO Division of Fisheries, Australia*
C.M. Wood, *McMaster University*
B. de Young, *Memorial University of Newfoundland*

NRC Research Press / Presses scientifiques du CNRC

Editor-in-chief / directeur général **Bruce P. Dancik**
Assistant Editor-in-chief / directeur général adjoint **Iain E.P. Taylor**

Journals Program / Programme des revues

(Fax / télécopieur : 613-952-7656; e-mail / adresse électronique : research.journals@nrc.ca;
URL: http://www.nrc.ca/cisti/journals/)
Director / directrice **Aldyth Holmes**
Business Manager / gestionnaire des affaires **Paul McClymont**
Managing Editor / rédactrice administrative **Cecily M. Pearson (613-993-9099)**
Publication Officer / agente de publication **Jennifer McColl**
Advertising / annonces publicitaires **Judy Heyman (613-993-0151)**
Reprints / tirés à part **Roxanne Landriault (613-990-2254)**
Subscriptions / abonnements **Helen Goulet (613-993-9084)**

© National Research Council of Canada 2000. World rights reserved. No part of this publication may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher, except as stated below.

The National Research Council of Canada grants **permission*** to individuals to make single copies of articles for personal use. Any person may reproduce short excerpts from articles in the journal for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal use, as stated above, is granted by the National Research Council of Canada provided that the copyright fee of $7.00 per copy is paid directly to CANCOPY, One Yonge Street, Suite 1900, Toronto, ON M5E 1E5, Canada.

*The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

© Conseil national de recherches du Canada 2000. Tous droits réservés. Il est interdit de reproduire, de sauvegarder ou de transmettre quelque partie de la présente publication dans quelque format ou de quelque façon sans le consentement écrit de l'éditeur, à l'exception des conditions suivantes.

Le Conseil national de recherches du Canada accorde aux particuliers le droit de faire des copies individuelles pour leur usage personnel. Toute personne peut reproduire de courts extraits des articles de la Revue pour autant que les droits moraux de l'auteur soient respectés et que la mention de provenance soit clairement indiquée. Il est de convenance de demander le consentement de l'auteur avant de faire mention de son travail.

On obtiendra l'autorisation de reproduire des articles à d'autres fins que l'usage personnel (tel qu'on l'a décrit ci-dessus) auprès du Conseil national de recherches du Canada pour autant que des redevances de droits d'auteurs de 7,00 $/copie soient versées au CANCOPY, 1, rue Yonge, bureau 1900, Toronto, ON M5E 1E5, Canada.

*La permission ci-dessus ne s'applique pas à la reproduction à grande échelle, telle que la reproduction aux fins de distribution générale, publicitaire ou promotionnelle, de compilation de nouveaux ouvrages collectifs ou de revente. Dans de tels cas, il faudra ratifier une entente particulière avec l'éditeur, le Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Subscription information

The *Canadian Journal of Fisheries and Aquatic Sciences* (ISSN 0706-652X) is published monthly by the National Research Council of Canada. 2000 annual subscription rates (second class postage) for Canada are $630CAN institutional and $191CAN personal, for US & foreign $630US institutional and $191US personal. For air mail add $150US to foreign rate. Canadian customers please include 7% GST (business no. 12149 1807 RT 0275). Subscriptions are entered for the calendar year. Remittances should be made payable to the Receiver General for Canada credit NRC. Price for regular single issues: institutional, $80.00; personal, $25.00. Supplements will be priced separately. Special rates apply to members of Canadian scientific and engineering societies if they subscribe through their respective societies.

There are no refunds on cancelled subscriptions once delivery has begun. Claims for missing issues should be made within four months (N. America) or six months (outside N. America) following the date of publication. The publisher expects to supply missing issues only when losses have occurred in transit and when the reserve stock will permit. For change of address and subscription enquiries: Subscription Office, NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada; tel: 613-993-9084; fax: 613-952-7656; e-mail: research.journals@nrc.ca.

Microform backfiles are available from UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A., and Micromedia Ltd., 20 Victoria St., Toronto, ON M5C 2N8, Canada.

All issues of the *Canadian Journal of Fisheries and Aquatic Sciences* since 1996 are available on the Web. Pricing information can be found at

http://www.nrc.ca/cisti/journals/

Renseignements sur l'abonnement

Journal canadien des sciences halieutiques et aquatiques (ISSN 0706-652X) est une publication mensuelle du Conseil national de recherches du Canada. Le prix de l'abonnement annuel au Canada (courrier de deuxième classe) est de 630 $CAN pour les organismes et de 191 $CAN pour les particuliers. À l'étranger (y compris les É.-U.), le prix de l'abonnement est de 630 $US pour les organismes et de 191 $US pour les particuliers. L'expédition par avion exige des frais de 150 $US en sus du prix d'abonnement à l'étranger. Les clients du Canada doivent ajouter la TPS de 7% (numéro d'entreprise 12149 1807 RT 0275). Les abonnements sont pour l'année civile. Tout paiement doit être adressé au Receveur général du Canada, crédit du CNRC. Le prix par numéro est de 80 $ pour les organismes et de 25 $ pour les particuliers. Le prix des hors-série varie. Les membres des sociétés canadiennes de sciences et de génie bénéficient de taux préférentiels en s'abonnant via leurs sociétés respectives.

Aucun remboursement ne sera accordé ni aucune annulation acceptée une fois que les livraisons auront commencé. Les réclamations de numéros manquants devront être soumises dans les quatre mois (Amérique du Nord) ou dans les six mois (à l'extérieur de l'Amérique du Nord) suivant la date de publication. L'éditeur ne fournira de numéros manquants que si le document a été perdu pendant l'expédition et que si le stock le permet. Adressez tout changement d'adresse ou toute question quant à l'abonnement au : Bureau des abonnements, Presses scientifiques du CNRC, Conseil national de recherches Canada, Ottawa, ON K1A 0R6, Canada; téléphone : 613-993-9084; télécopieur : 613-952-7656; adresse électronique : research.journals@nrc.ca.

On peut obtenir les fichiers rétrospectifs sur **microforme** auprès de UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, É.-U., et auprès de Micromedia Ltée, 20 rue Victoria, Toronto, ON M5C 2N8, Canada.

À partir de 1996, tous les numéros de *Journal canadien des sciences halieutiques et aquatiques* sont accessibles sur le Web. On trouvera des renseignements sur les tarifs à

http://www.nrc.ca/cisti/journals/rjfr.html

RAPID COMMUNICATIONS / COMMUNICATIONS RAPIDES

Pelagic fishes and the cod recruitment dilemma in the Northwest Atlantic

D.P. Swain and A.F. Sinclair

Abstract: Like most other stocks of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic, cod in the southern Gulf of St. Lawrence declined to low abundance in the early 1990s. Recovery has been slow in contrast with the rapid recovery from similar levels of abundance in the mid-1970s. This difference reflects remarkably high prerecruit survival of cod in the earlier period of low abundance rather than unusually poor survival in the 1990s. The period of high prerecruit survival of cod coincided with the collapse of herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) stocks resulting from overfishing. These pelagic fishes are potential predators or competitors of the early life history stages of cod. We report a strong negative relationship between the biomass of these pelagic fishes and recruitment rate of southern Gulf cod. This is consistent with the recent suggestion that the success of large predatory fishes may depend on "cultivation" effects in which the adults crop down forage fishes that are predators or competitors of their young. Our results also point to the possibility of a triangular food web involving cod, seals, and pelagic fishes, making it difficult to predict the effect of a proposed cull of seals on the recovery of cod.

Résumé : Comme la plupart des autres stocks de morue (*Gadus morhua*) de l'Atlantique Nord-Ouest, le stock de morue du sud du golfe du Saint-Laurent a atteint un faible niveau au début des années 90. Son rétablissement a été lent comparativement au rétablissement rapide qui s'est produit à partir de niveaux d'abondance similaires au milieu des années 70. Cette différence s'explique par le taux de survie remarquablement élevé des pré-recrues dans la période antérieure de faible abondance plutôt que par le taux de survie inhabituellement bas dans les années 90. La période de fort taux de survie des pré-recrues a coïncidé avec l'effondrement des stocks de hareng (*Clupea harengus*) et de maquereau (*Scomber scombrus*) dû à la surpêche. Ces poissons pélagiques sont des prédateurs ou des compétiteurs potentiels de la morue dans les premiers stades de sa vie. Nous avons relevé une forte relation négative entre la biomasse de ces poissons pélagiques et le taux de recrutement de la morue du sud du golfe. Cette observation va dans le sens de l'idée récemment avancée suivant laquelle le succès des grands poissons prédateurs peut dépendre d'un « effet cultural », dû au fait que les adultes prélèvent des poissons fourrage qui sont des prédateurs ou des compétiteurs de leurs jeunes. Nos résultats suggèrent aussi l'existence possible d'un réseau alimentaire triangulaire impliquant la morue, les phoques et des poissons pélagiques, ce qui rend difficile la prévision de l'effet d'une moisson sélective des phoques envisagée sur le rétablissement de la morue.

[Traduit par la Rédaction]

Introduction

Abundance of many Northwest Atlantic cod (*Gadus morhua*) populations declined to low levels in the early 1990s, resulting in moratoria on directed fishing for these stocks. Recovery of these stocks has been slow despite severe restrictions on fishing pressure. In contrast with this slow recovery, these cod populations recovered rapidly from declines to low abundance in the mid-1970s (Sinclair 1996), even though commercial fishing continued during the earlier period of low abundance. The recent failure of depressed cod stocks to recover from low abundance despite restricted fishing mortality has been termed a "recruitment dilemma" (FRCC 1999).

In this report, we describe a strong negative relationship between pelagic fish biomass and recruitment rate of cod in the southern Gulf of St. Lawrence. We suggest that an effect of pelagic fishes on cod recruitment success may be one cause of the apparent dilemma. We also discuss implications of such an effect for management action that has been proposed to accelerate the recovery of cod in the Northwest Atlantic, namely a cull of seal populations in this area.

What is the dilemma?

Has recruitment during the recent period of low abundance been unusually low, or was it unusually high during

Received January 19, 2000. Accepted May 3, 2000. J15533

D.P. Swain.[1] Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, NB E1C 9B6, Canada.
A.F. Sinclair. Department of Fisheries and Oceans, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9R 5K6, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: swaind@dfo-mpo.gc.ca

Can. J. Fish. Aquat. Sci. **57**: 1321–1325 (2000)

the earlier low-abundance period? An index of juvenile or prerecruit survival can be calculated by dividing R, the number of recruits, by S, the spawning stock biomass that produced them. We calculated this index for the southern Gulf of St. Lawrence cod stock. We used two independent time series of recruitment and spawning biomass, one based on the mean catch per tow in annual research vessel surveys and one estimated using sequential population analysis (SPA)[2]. The survey recruitment index was based on catches of 2- and 3-year-old cod; the SPA index was the estimated abundance of 3-year-old cod. Both the SPA- and survey-based indices indicate that R has not been unusually low in recent years given the recent low values of S (Fig. 1A), with estimates of prerecruit survival in the 1990s comparable with or above those that persisted throughout the 1950s, 1960s, and early 1970s (Fig. 1B). Instead, prerecruit survival was remarkably high from the mid-1970s to the early 1980s. Thus, at least for southern Gulf cod, the dilemma is not posed by unusually low recruitment in recent years but rather by the unusually high recruitment rate in the earlier period of low abundance. A similar pattern of remarkable prerecruit survival in the mid- to late 1970s is also evident in most other cod stocks in the Northwest Atlantic (Sinclair 1996).

Pelagic fishes and cod recruitment

An effect of pelagic fish abundance on cod recruitment has often been suggested. Most studies have focussed on top-down control by pelagic fishes through predation on cod eggs and larvae, though bottom-up control through competition with early life history stages of cod is also a possibility. Heavy predation on cod eggs by herring and sprat has been demonstrated in the Baltic Sea, leading to the suggestion that the recovery of the Baltic cod stock from low population sizes may be hampered by recent increases in the abundance of these pelagic fishes (e.g., Köster and Möllmann 2000). Cod eggs have also been reported from herring stomachs in the North Sea, where an increase in the recruitment of cod and other gadoids coincided roughly with the collapse of herring (*Clupea harengus*) and mackerel stocks (*Scomber scombrus*) resulting from overfishing (Daan et al. 1994). Lett (1980), noting that mackerel consume large quantities of fish eggs and larvae, proposed a negative effect of mackerel biomass on cod recruitment in the southern Gulf of St. Lawrence. Similarly, Paz and Larrañeta (1992) suggested that cod year-class strength on the Grand Bank is influenced by mackerel predation on cod eggs and larvae.

To develop a better understanding of the factors influencing cod spawning success in the southern Gulf of St. Lawrence, we have examined a variety of biotic and abiotic factors that may affect cod recruitment. On the basis of these preliminary analyses, the unusually high recruitment rate of southern Gulf cod from the mid-1970s to the early 1980s did not appear to be attributable to climatic forcing or characteristics of the spawning stock but could be accounted for by a negative effect of herring and mackerel on cod spawning success.

Evidence that herring and mackerel have affected cod recruitment in the southern Gulf of St. Lawrence

Herring and mackerel comprise the major portion of the pelagic fish biomass in the southern Gulf of St. Lawrence (Winters 1976). We used estimates of the biomass of spring- and fall-spawning herring aged 2 years and older (Fig. 1C) and of mackerel spawning biomass (Fig. 1D) based on SPA[2]. Estimates of mackerel biomass were for both the southern stock component spawning in waters off New England and the northern component spawning primarily in the southern Gulf. The two components are believed to be of similar size (F. Grégoire, Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, 800 route de la Mer, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada, personal communication), and we constructed an index of pelagic fish biomass in the southern Gulf by adding the herring biomass to half the mackerel biomass.

Estimated herring biomass in the southern Gulf exceeded 1 000 000 t throughout the 1960s but declined precipitously in the late 1960s and early 1970s (Fig. 1C) when landings rose tenfold with the development of a new purse-seine fishery (Messieh 1991). Mackerel biomass was also high in the late 1960s and early 1970s (Fig. 1D) but then also declined sharply following the rapid expansion of a new distant-water trawl fishery in the late 1960s (Anderson and Paciorkowski 1980). The period of exceptionally low herring and mackerel biomass that resulted from these population collapses coincided closely with the period of remarkable prerecruit survival of cod (Fig. 1).

We tested for an effect of herring and mackerel biomass on the stock–recruitment relationship of southern Gulf cod. We assumed a Ricker relationship with lognormal error. We fit the relationship using linear regression, with $\log_e(R/S)$ as the dependent variable and S and the estimates of herring and mackerel biomass in the year of spawning as the independent variables. Durbin-Watson tests rejected the hypothesis of zero lag-1 autocorrelation in the residuals from the ordinary least squares regressions in all cases (at $\alpha < 0.1$). Thus we simultaneously computed maximum likelihood estimates of the regression parameters and the first-order autoregressive parameter for the error. Analyses were conducted for two time periods: 1971–1994, when both survey and SPA estimates of S and R were available and biomass estimates are likely to be the most reliable for all three species, and 1963–1994, the entire period with SPA estimates for all three species.

The recruitment rate of southern Gulf cod was highest when both S and pelagic fish biomass were low (Fig. 2). Regressions of recruitment rate on S were improved substantially by the addition of herring, mackerel, or total pelagic biomass as a covariate, with parameter estimates for the covariate in the predicted negative direction (Table 1). Fits of regression models including S alone were relatively weak, with strongly autocorrelated error terms (suggesting the action of factors not included in the models). In both the SPA and survey analyses for the 1971–1994 period, addition of either herring, mackerel, or total pelagic biomass increased

---

[2] Data are from various stock assessment documents (and Winters (1976) for herring before 1969), a list of which may be obtained from the authors upon request.

Fig. 1. Cod stock and recruitment and pelagic fish biomass in the southern Gulf of St. Lawrence. (A) Cod spawning stock biomass, estimated by SPA (sequental population analysis, solid line) or based on catch per tow in the annual research vessel survey (dotted line). (B) Indices of cod prerecruit survival, calculated as the ratio of recruits (*R*) divided by spawning stock biomass (*S*), estimated by SPA (solid line) or survey catch per tow (dotted line). (C) Herring biomass for ages 2 years and older. (D) Mackerel spawning stock biomass.

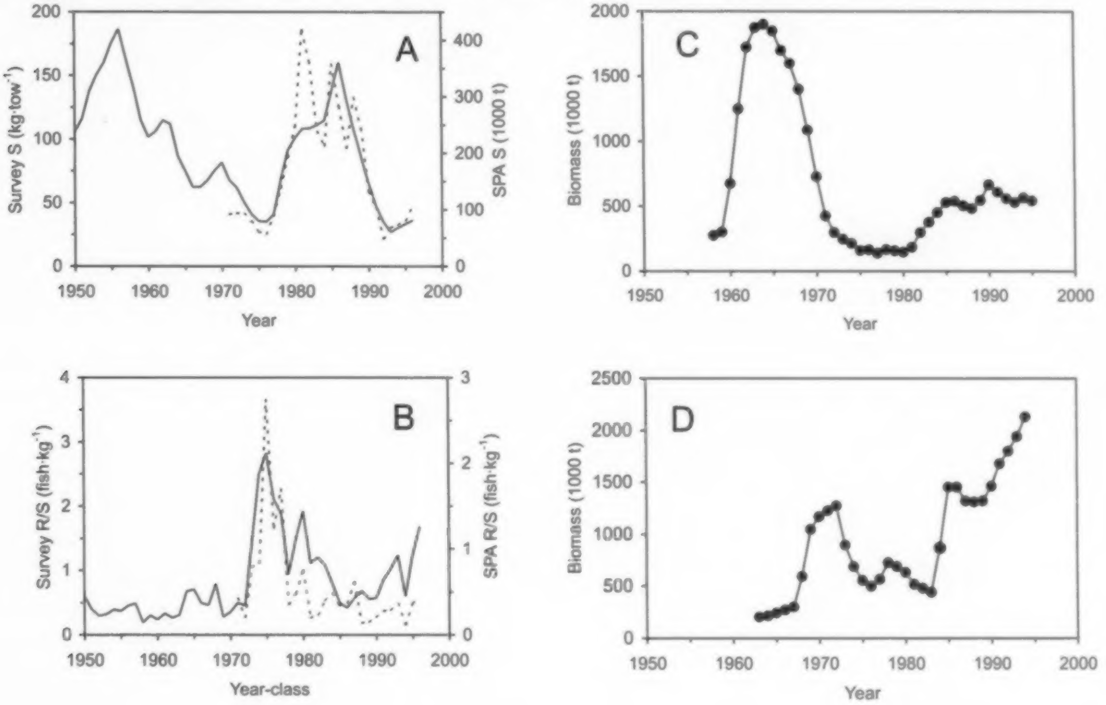


Table 1. Stock–recruitment analysis for southern Gulf of St. Lawrence cod, incorporating effects of herring and mackerel biomass on recruitment success ($\log_e R/S$).

Covariate	None		Herring		Mackerel		Total pelagic	
(A) Survey, 1971–1994								
b_S	–0.5544	(0.068)	–0.4025	(0.042)	–0.5522	(0.0042)	–0.4891	(0.0091)
b_C	—	—	–0.6076	(0.0051)	–0.6940	(0.0007)	–0.6827	(0.0009)
$A(1)$	0.6015	(0.013)	0.2209	(0.37)	0.1863	(0.43)	0.1887	(0.43)
R^2	0.18		0.43		0.53		0.52	
R_T^2	0.39		0.52		0.60		0.59	
(B) SPA, 1971–1994								
b_S	–0.5998	(0.094)	–0.3833	(0.054)	–0.5720	(0.0038)	–0.4969	(0.0094)
b_C	—	—	–0.7195	(0.0017)	–0.7435	(0.0003)	–0.7816	(0.0003)
$A(1)$	0.7471	(0.0002)	0.4168	(0.064)	0.4160	(0.053)	0.4286	(0.051)
R^2	0.15		0.50		0.57		0.58	
R_T^2	0.58		0.71		0.75		0.75	
(C) SPA, 1963–1994								
b_S	–0.6052	(0.028)	–0.4504	(0.045)	–0.6247	(0.017)	–0.4221	(0.0085)
b_C	—	—	–0.4925	(0.048)	–0.5700	(0.039)	–0.7393	(0.0001)
$A(1)$	0.7503	(0.0001)	0.5763	(0.0017)	0.7961	(0.0001)	0.4006	(0.032)
R^2	0.17		0.27		0.29		0.53	
R_T^2	0.59		0.62		0.65		0.72	

Note: b_S and b_C are the standard partial regression coefficients for *S* (cod spawning stock biomass) and for a covariate *C* (either herring, mackerel, or total pelagic biomass). $A(1)$ is the first-order autoregressive parameter for the error. Significance levels are given in parentheses. R^2 gives the proportion of the variation explained by *S* and *C* after transformation to adjust for the estimated autocorrelation. R_T^2 gives the proportion of the variation accounted for by the full model, including the autoregressive error process.

R^2 values by a factor of 2–4 and greatly reduced autocorrelation in the error term. For the 1963–1994 period, model improvement resulting from the addition of a term for either herring or mackerel biomass was less substantial, though either effect remained significant (two-sided $P < 0.05$). The effect of total pelagic biomass remained highly significant ($P < 0.0001$) for the 1963–1994 period, tripling the R^2 value for the regression and substantially reducing the significance of autocorrelation in the error. In all cases, addition of pelagic fish biomass to the stock–recruitment model revealed that the density dependence of prerecruit survival was also highly significant, with high survival at low spawning stock biomass. However, in most analyses, prerecruit survival was even more strongly related to measures of pelagic fish biomass than to cod spawning stock biomass (Table 1, Fig. 2).

Discussion

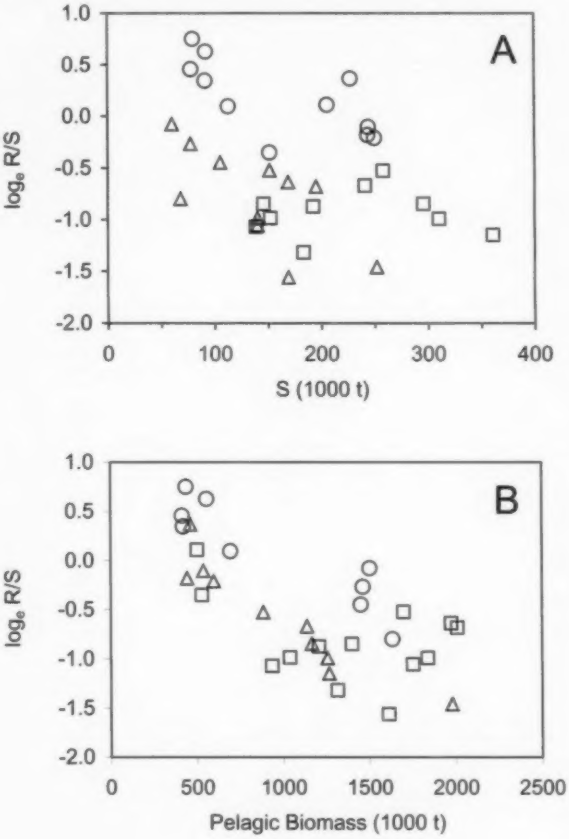
Our analyses suggest a strong negative effect of pelagic fish biomass on cod recruitment success in the southern Gulf of St. Lawrence, an hypothesis proposed by Lett (1980) some 20 years ago. As in any observational study, we cannot exclude the possibility that this correlation between cod prerecruit survival and pelagic fish biomass is spurious. However, it provides a reasonable explanation (and the only explanation yet apparent) for the remarkable recruitment rate of southern Gulf cod between the mid-1970s and the early 1980s. Similar effects of pelagic fishes on cod recruitment have been proposed for the Baltic Sea (e.g., Köster and Möllmann 2000), the North Sea (e.g., Daan et al. 1994), and the Grand Bank (Paz and Larrañeta 1992). Lett's (1980) original proposal for the southern Gulf was based on data for the 1954–1972 period. Our analyses provide independent support for this proposal based on more recent data, making this one of few cases where a correlation with recruitment rate has stood the test of time.

Although predation by pelagic fishes on cod eggs and larvae has been demonstrated in other areas (e.g., Köster and Möllmann 2000), there are no data available to examine the extent of such predation in the southern Gulf. However, the opportunity for significant predation by herring and mackerel on eggs and larvae of southern Gulf cod clearly exists. Spawning by cod in the southern Gulf occurs from May to early July, and eggs and larvae are widely distributed throughout the southern Gulf in June and July. This is a period of heavy feeding by herring and mackerel in the southern Gulf, following spawning in spring and early summer. In the Baltic Sea, where predation by pelagic fishes on cod eggs has been intensively studied, estimated consumption of cod eggs by herring and sprat often exceeded estimated egg abundance (Köster and Möllmann 2000). In the southern Gulf, the ratio of pelagic fish biomass to cod spawning stock biomass exceeds that reported for the Baltic (D. P. Swain, unpublished data), suggesting that the potential for a substantial impact of pelagic fish predation on cod eggs exists in this area also.

The data since 1963, when biomass estimates are available for both pelagic fishes, support the hypothesis of a negative effect of pelagic fish biomass on cod recruitment success. The scant data available for earlier years do not provide additional support for this hypothesis (though biomass estimates from SPA must be viewed with caution for these earlier years, when sampling of the landings was less extensive and the abundance indices needed to verify SPA assumptions are lacking). Cod prerecruit survival appeared to be unremarkable in the late 1950s even though herring biomass appeared to be very low in 1958 and 1959 (Fig. 1). However, it is likely that many factors influence early survival of cod and it may be that other factors limited cod recruitment success in the late 1950s. For example, prerecruit survival appears to be lower at high levels of cod biomass (Table 1), and cod biomass was at a high level in the mid- to late 1950s (Fig. 1). Thus, the exceptional recruitment rate of southern Gulf cod in the mid-1970s appears to reflect the combined effects of very low biomasses for both cod and pelagic fishes. Recruitment rates were lower in the early 1990s when cod biomass was low but pelagic fish biomass was high and in the late 1950s when pelagic fish biomass appeared to be low but cod biomass was high.

Recruitment rate appeared to be unusually high for many

Fig. 2. Relationships between cod recruitment rate and (A) cod spawning stock biomass S or (B) pelagic fish biomass in the southern Gulf of St. Lawrence. Different symbols are used for different levels of pelagic fish biomass (A) or cod spawning stock biomass (B) (low, circles; medium, squares; high, triangles). Data are for 1963–1994 and are estimated by SPA.



Northwest Atlantic cod populations in the mid- to late 1970s (Sinclair 1996). Can reduced biomasses of pelagic fishes provide an explanation for the high recruitment success in these other populations during this period? Although spawning by the northern population of mackerel in the Northwest Atlantic is principally in the southern Gulf of St. Lawrence, these fish feed over a wider area, and the collapse of this population in the mid- to late 1970s may have contributed to high prerecruit survival of cod in other areas during this period, as suggested by Paz and Larrañeta (1992) for the Grand Bank. Similarly, the rapid development of purse-seine fisheries for herring in the late 1960s resulted in declines in many Northwest Atlantic herring stocks in the 1970s (Messieh 1991).

Our results are consistent with the suggestion by Walters and Kitchell (2000) that the success of many of the large commercially important fishes depends on "cultivation" effects in which the adults crop down forage fishes that are predators or competitors of their early life history stages. A consequence of these cultivation effects can be delayed depensatory decreases in juvenile survival when adult abundance is severely depleted by fishing. However, in our case, it would appear that the corollary may have been the case, with fisheries-induced collapses of pelagic fishes improving cod prerecruit survival and aiding the recovery of cod in the late 1970s.

Our results could also have implications for the recommendation that seal herds off Atlantic Canada should be reduced to promote cod stock recovery (FRCC 1999). It has been suggested that large increases in seal abundance have resulted in increased predation on cod, thus delaying stock recovery. However, pelagic fishes like herring appear to be an important component of seal diets (e.g., Bowen et al. 1993). These results point to the possibility of a triangular food web involving cod, seals, and pelagic fishes. Manipulation of such food webs can lead to unexpected results (Bax 1998). Seals may have a direct negative effect on cod recruitment through predation on prerecruit cod. They also may have an indirect positive effect through predation on pelagic fishes, possible predators of early life history stages of cod, so that reductions in the seal herds may not lead to improved recruitment of cod. Bax (1998) emphasizes the danger of concentrating only on direct predation interactions when there are indirect interactions with the potential to reverse the direct effects. Our results suggest that a better understanding of the interactions between cod, seals, and pelagic fishes is needed before it will be possible to predict the effect of a seal cull on cod abundance.

Acknowledgements

We thank François Grégoire for information on mackerel in the Northwest Atlantic and Jim Irvine, Ghislain Chouinard, Gerald Chaput, Ransom Myers, and an anonymous referee for helpful comments on the manuscript. We extend our apologies to G.A. Chouinard, S.H. Clark, R.R. Claytor, L. Cleary, and their co-authors, whose stock assessment documents we were unable to cite due to reference limits.

References

Anderson, E.D., and Paciorkowski, A.J. 1980. A review of the Northwest Atlantic mackerel fishery. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **177**: 175–211.

Bax, N.J. 1998. The significance and prediction of predation in marine fisheries. ICES J. Mar. Sci. **55**: 997–1030.

Bowen, W.D., Lawson, J.W., and Beck, B. 1993. Seasonal and geographic variation in the species composition and size of prey consumed by grey seals (*Halichoerus grypus*) on the Scotian Shelf. Can. J. Fish. Aquat. Sci. **50**: 1768–1778.

Daan, N., Heessen, H.J.L., and Pope, J.G. 1994. Changes in the North Sea cod stock during the twentieth century. ICES Mar. Sci. Symp. **198**: 229–243.

Fisheries Resource Conservation Council (FRCC). 1999. 1999 conservation requirements for the Gulf of St. Lawrence groundfish stocks and cod stocks in Divisions 2GH and 3Ps. April 1999 report from the Fisheries Resource Conservation Council to the Minister of Fisheries and Oceans, Canada.

Köster, F.W., and Möllmann, C. 2000. Trophodynamic control by clupeid predators on recruitment success in Baltic cod? ICES J. Mar. Sci. **57**. In press.

Lett, P. F. 1980. A comparative study of the recruitment mechanisms of cod and mackerel, their interaction, and its implication for dual stock assessment. Can. Tech. Rep. Fish. Aquat. Sci. No. 988.

Messieh, S.N. 1991. Fluctuations in Atlantic herring populations in the Northwestern Atlantic, with particular emphasis on the Gulf of St. Lawrence stocks. *In* Long-term variability of pelagic fish populations and their environment. *Edited by* T. Kawasaki, S. Tanaka, Y. Toba, and A. Taniguchi. Pergamon Press, Ltd., Oxford. pp. 155–163.

Paz, J., and Larraneta, M.G. 1992. Cod year-class variations and abundance of other commercial fish in NAFO Divisions 3NO. J. Northwest Atl. Fish. Sci. **14**: 129–134.

Sinclair, A. 1996. Recent declines in cod species stocks in the Northwest Atlantic. NAFO (Northwest Atl. Fish. Org) Sci. Counc. Stud. **24**: 41–52.

Walters, C.J., and Kitchell, J.F. 2000. Cultivation/depensation effects on juvenile survival and recruitment: a serious flaw in the theory of fishing? Can. J. Fish. Aquat. Sci. **57**. In press.

Winters, G.H. 1976. Recruitment mechanisms of southern Gulf of St. Lawrence herring (*Clupea harengus harengus*). J. Fish. Res. Board Can. **33**: 1751–1763.

Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology[1]

J.-M. Roussel, A. Haro, and R.A. Cunjak

Abstract: A new method for tracking small fishes in shallow streams based on passive integrated transponder (PIT) technology, using a portable reading unit, was investigated. The device consists of a chest-mounted palmtop computer, a reader, and a 12-V battery enclosed in a backpack, connected to a 60-cm-diameter coil antenna mounted on a 4-m-long pole. The method was field tested with wild Atlantic salmon, *Salmo salar*, parr using transponders 23.1 mm long and 3.9 mm in diameter surgically implanted in the peritoneal cavity of the fish. Laboratory experiments indicated no posttagging mortality for fish > 84 mm in fork length and no tag loss when sutures were used. In the field, tag detection distance was up to 1 m. While moving the antenna above the stream surface, the operator could locate a fish's position to within a square metre. Experiments indicated that more than 80% of tagged parr, on average, were detected by the reader. The technique is a useful alternative to standard radiotelemetry in small-scale environments because PIT tags can be implanted in smaller-bodied fishes and fine-scale movements of individuals can be studied. It can be applied to address numerous questions in the fields of animal behaviour, habitat use, and population dynamics.

Résumé : S'appuyant sur la technologie PIT (passive integrated transponder), une nouvelle méthode est proposée pour le pistage de poissons de petite taille en rivière peu profonde. L'unité portable de détection se compose d'un ordinateur de poche, d'une batterie de 12 V et d'un module de lecture, connecté à un câble enroulé formant une antenne de 60 cm de diamètre. L'ordinateur est porté à la ceinture, le lecteur et la batterie dans un sac à dos, et l'antenne que manipule l'opérateur est fixée au bout d'une perche de 4 m de long. La méthode a été testée in situ sur des juvéniles de saumon atlantique, *Salmo salar*, avec des transpondeurs de 23,1 mm de long et 3,9 mm de diamètre implantés chirurgicalement dans la cavité péritonéale. Des tests en laboratoire ont indiqué l'absence de mortalité pour les poissons de taille supérieure à 84 mm (longueur fourche), aucun rejet du transpondeur n'étant enregistré lorsqu'une suture est faite. Sur le terrain, le poisson peut être détecté jusqu'à un mètre de distance : en balayant de l'antenne la surface de l'eau, l'opérateur peut déterminer sa position avec une précision de l'ordre du mètre carré. Les essais indiquent que, en moyenne, plus de 80% des poissons sont détectés. Cette méthode est une alternative utile à la radio-télémétrie classique, car des individus de petite taille peuvent être équipés pour suivre des déplacements de courte amplitude. Elle peut permettre d'aborder de nombreuses questions relatives au comportement du poisson, à son habitat et à la dynamique de population d'une espèce.

Introduction

Studying movement and habitat use by individual fish in streams is usually required to address the functional mechanisms in fish–habitat relationships. Although radiotelemetry is commonly used for large-bodied and mobile fishes, the size of the transmitter and its antenna preclude any study on small individuals. For salmonids, Adams et al. (1998) noted an impact on swimming ability of juvenile chinook salmon (*Oncorynchus tshawytscha*) and consequently recommended against implanting transmitters in fish < 120 mm in fork length (FL). In the past decade, the passive integrated transponder (PIT) technology has been developed as a novel method for individually tagging fish (Prentice et al. 1990). The tag consists of an integrated circuit chip, capacitor, and antenna coil encapsulated in a glass cylinder, and its operation requires an external energy source. An electromagnetic field generated by the reading unit induces current in the an-

Received April 6, 2000. Accepted May 3, 2000.
J15696

J.-M. Roussel.[2] Department of Biology, University of New Brunswick, Bag service 45111, Fredericton, NB E3B 6E1, Canada.
A. Haro. S.O. Conte Anadromous Fish Research Center, Biological Resources Division, U.S. Geological Survey, 1 Migratory Way, P.O. Box 796, Turners Falls, MA 01376, U.S.A.
R.A. Cunjak. Department of Biology and Faculty of Forestry and Environmental Management, University of New Brunswick, Bag service 45111, Fredericton, NB E3B 6E1, Canada.

[1] Contribution No. 58 of the Catamaran Brook Habitat Research Project.

[2] Author to whom all correspondence should be sent at the following address: INRA UMR EQHC, Laboratoire d'Ecologie Aquatique, 65 rue de Saint-Brieuc, F-35042 Rennes, France. e-mail: roussel@roazhon.inra.fr

tenna coil that energizes the integrated circuit, which transmits its signal to the reader. Tag detection range depends on the size and orientation of the tag antenna coil and the overall size of the tag. Typical length dimensions of commercially available implantable PIT tags vary from 11 to 32 mm; therefore, PIT tags can be implanted in the peritoneal cavity of small fish, such as young salmonids. Focusing on movement of fish in running waters, recent advancements have led to the development of stationary and automatic reading units, such as flat-bed antennae (Armstrong et al. 1996) or electronic weirs (Castro-Santos et al. 1996; Barbin-Zydlewski et al., U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, Longview, Wash., unpublished data). With such technological improvements, it has become possible to design mobile reading units for monitoring small-scale movements of fish in shallow streams. In this paper, we describe the use of PIT technology to study diel movements and habitat use by age-1 and older Atlantic salmon parr (*Salmo salar*), with special regard for testing its efficiency to detect and locate individuals in two streams.

Materials and methods

Tagging method and laboratory experiment

The PIT tags used for the tests were 23.1 mm long, 3.9 mm in diameter, weighed 0.6 g in air, and were manufactured by Texas Instruments (TIRIS series 2000; RI-TRP-WRHP). Initially, 33 hatchery-reared Atlantic salmon parr 64–94 mm FL were used to determine size-dependant effects of implanting PIT tags. Fish were held in two indoor Fiberglas tanks (100 L, 75 cm in diameter, 25 cm deep) at the University of New Brunswick. Artificial fluorescent lighting was provided, beginning at 5:00 and ending at 20:00. Each tank was supplied with well water at 12°C–13.5°C with 68%–91% of oxygen saturation. The water flow was maintained at the minimum amount needed to produce a circular current in the tanks. Fish were fed on standard-grade food pellets at a rate of at least 3.3% of body weight per day, but no food was delivered 24 h before and after tagging. For surgery, fish were individually anesthetized in a solution of phenoxyethanol (0.03%). A 4-mm-long incision was made on the midventral line starting 15–20 mm anterior to the pelvic girdle, deep enough to penetrate the peritoneum. The transponder was then implanted, gently pushing it posteriorly within the cavity. The incision was closed with one simple, interrupted non-absorbable surgical suture (Ethicon silk braided, 6-0 taper C-1 needle) in the middle of the incision (Summerfelt and Smith 1990). The suture was used for only 15 of the 33 parr tagged. Antibacterial ointment (Polymyxin B) was applied to the incision to prevent infection.

Thirty-two days after tagging, seven parr had died (21.2%). All mortalities were parr < 84 mm FL. Among the 26 surviving parr, five had lost their tag (15.2%); however, no tag loss was recorded when sutures were used. The incision was well healed for each of the 21 remaining tagged parr.

Portable reading unit

The portable reading unit was constructed using a commercially available radio frequency identification system (Texas Instruments TIRIS S-2000). The system consisted of a half-duplex reader module (TIRIS RI-RFM-008) operating at 134.2 kHz, connected to a control module (TIRIS RI-CTL-MB2A). The reader/control modules were powered by a rechargeable 12-V DC lead–acid battery that provided approximately 2 days of continuous run time on a single charge. Both modules were housed in a plastic box 25 cm long x 15 cm wide x 15 cm deep. The instrumentation box and battery were enclosed in a small rigid-frame backpack. The reader module was connected to an open loop inductor antenna that both generated an energizing electromagnetic field and received transmitted signals from the tag. The antenna was constructed using 12-gauge insulated THHN multistrand wire; the wire was wound to form a 60-cm-diameter inductor coil (6 loops), and its two extremities (5 m in length) were connected to the reading module. The inductor coil was tied to a square PVC tubing frame and mounted on a wooden pole 4 m in length so as to be rigid and to facilitate the handling of the antenna. A bank of tuning capacitors (TIRIS RI-ACC-008) was connected to the circuit between the wand and the reader module and was housed in the backpack; selection of combinations of capacitors allowed the antenna circuit to be tuned to the resonant frequency. A palmtop computer (Hewlett Packard HP200LX) was connected to the control module via an RS-232 serial cable. A custom software program (written in BASIC language) continuously displayed and logged tag code data sent from the control module via the RS-232 interface, along with date and time information. The palmtop computer was housed in a transparent plastic box and carried outside of the backpack (usually chest-mounted) so that the operator could easily read detected tag codes as they were displayed. A piezoelectric buzzer was also connected to an alarm circuit on the control module, which sounded a loud tone whenever a tag was detected in the antenna field. The total weight of the complete system was approximately 12 kg. The design of the portable reading unit was adapted from Barbin-Zydlewski et al. (U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, Longview, Wash., unpublished data).

The detection range (measured as the distance between the plane of the antenna loop and the tag, while holding the antenna horizontally above a PIT tag) varied with the orientation of the tag: up to 70 cm when the tag was horizontal and up to 100 cm when the tag was vertical. The tag was easily read in the water and within the substrate (up to 20 cm deep in a cobble–gravel mixture) without consequence to detection efficiency. Any transponder that entered into the antenna field for at least 100 ms was detected. The efficiency of the portable device to detect and locate a fish was evaluated in two shallow-water tributaries of the Little South West Miramichi River (N.B), Otter Brook, and Catamaran Brook, each offering natural but contrasting habitat conditions for Atlantic salmon parr.

Field test of the efficiency of the device

Otter Brook is a small second-order tributary. Channel width ranges from 2 to 4 m and mean gradient is 1.2%. The brook is characterised by the presence of woody debris in the channel, rocky substrate, and a complete canopy of overhanging trees and branches. A 25-m-long riffle-run sequence (mean width 3.1 m) was enclosed with two barrier nets (5-mm mesh) set up across the channel. Based on 69 evenly spaced measurements in the site, mean and maximum water depths were 12 cm and 33 cm, respectively.

On 23 June 1999, Atlantic salmon parr were captured in the site using a backpack electrofisher (Smith-Root type 12, 500 V, 60 Hz). According to the laboratory findings, PIT tags were implanted in 23 parr > 84 mm FL (representing 3.8–9.7% of the fish's body weight), and a suture was used for each individual. Fish were then released back into the site, between the two barrier nets. On 30 June and 1 July 1999, tagged fish were tracked six times during daylight hours. When tracking, the operator walked in an upstream direction on the stream bank, from one barrier net to the other, moving the antenna just above the stream surface from one bankside to the other. The number of fish detected per tracking varied from 17 (73.9%) to 21 (95.6%). On average, the efficiency of the reader for detecting tagged parr was 81.9%.

The second test was performed in the lower reach of Catamaran Brook, where the mean gradient is 0.6% and the channel is 6–13 m wide. Since 1990, a fish counting fence at the mouth of the brook has been operated to monitor Atlantic salmon movement (Hardie et al. 1998). Approximately 50 m upstream of the counting fence, a 72-m-long site (7.5 m wide on average) was marked out with flags set every metre on both banks. The site consisted of a riffle-run succession where mean water depth was 21 cm (estimated from 360 measurements); maximum depth was 79 cm. The study site was electrofished on 10 July, 12 July, and 25 July 1999. PIT tags (representing 3.2–10.0% of the fish's body weight) were implanted in 46 fish > 84 mm FL, using the same procedure as for Otter Brook (above). As no barrier nets were used here, the fish were free to move from the site after release.

In total, five fish were trapped at the counting fence within 48 h after tagging. On 2 August 1999, three individuals were detected within a 50-m zone outside the site boundaries. One of the PIT tags was found on the stream bank of the site; the fish carrying the tag was apparently killed by a mink, *Mustella vison*. The efficiency of the reader was calculated, therefore, assuming that a maximum of 37 fish were present in the site. From 2–7 August 1999, the tagged parr were tracked in the site six times during daylight and six times at night. The operator waded upstream, moving the antenna just above the stream surface and from one bankside to the other. The antenna was moved underwater only in the deepest habitats (>60 cm) to detect fish on the streambed. The number of fish recorded varied from 31 (83.8%) to 34 (91.9%) during daylight hours (87.3% in average) and from 32 (86.5%) to 34 (91.9%) at night (88.3% in average).

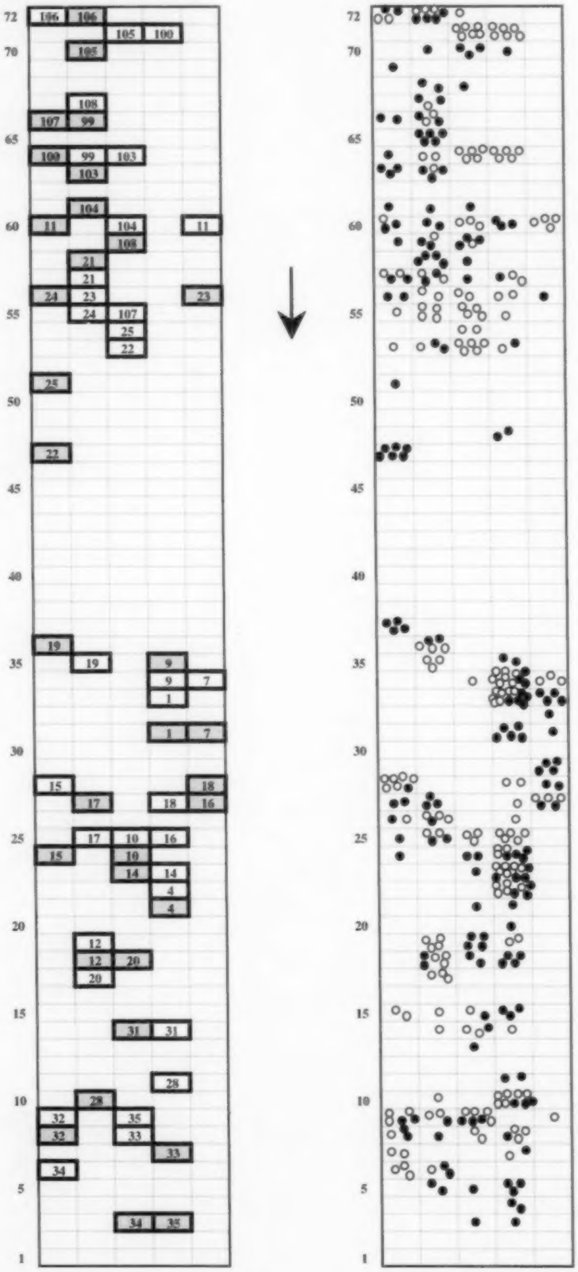
Tracking surveys were comparatively easier in Catamaran Brook than in Otter Brook as no woody debris or overhanging trees and branches hampered the operator when handling the antenna. The few parr not recorded during a tracking were likely holding stations in microhabitat not accessible to the operator. Indeed, access to all available in-stream habitats was the major limitation of the method.

Consecutive trackings in Catamaran Brook did not provide any evidence of tag loss or fish mortality after tagging as each PIT tag was detected in several locations within the site during the experiment. Preliminary data for the growth of tagged parr in this study indicate that except for one individual, fish grew from 1 to 8 mm during the period of July to September. This corresponds to the average growth typically observed during mid-summer for parr > 84 mm in Catamaran Brook (R.A. Cunjak, unpublished data). It appears, therefore, that implanting the transponder did not induce a negative effect on fish growth as also noted for other salmonid species under different conditions (Prentice et al. 1990; Ombredane et al. 1998).

Potential applications and interests

Once a fish was detected in Catamaran Brook, its position in the channel was plotted in accordance with the flags along the banks and by visually dividing the stream width into 5

Fig. 1. Location of PIT-tagged Atlantic salmon parr in Catamaran Brook, as determined by a portable reader. Numbers from 1 to 72 represent metres along the site, whereas the channel width is divided into five equal-sized cells. The arrow indicates the flow direction. Left: results from two consecutive tracking surveys, during daytime (open cell) and the following night (shaded cell). Numbers in cells indicate tag codes that identify and locate individuals. Right: spatial distribution of fish within the site, resulting from the six daytime (open circles) and the six nighttime (solid circles) trackings.



cells. Occasionally at night, direct observations of fish detected in low velocity habitats along the stream edge confirmed that the operator was able to locate the fish to within a square metre. Results from the successive tracking surveys in Catamaran Brook (Fig. 1) demonstrated how the mobile reader could be used to track parr implanted with PIT tags in shallow-water streams.

Further improvements are needed to increase the detection range of the portable reading unit. For the moment, the new tracking method is limited to shallow-water streams (<1 m deep) and to species that allow the operator to get fairly close. However, it represents a useful alternative to standard radiotelemetry because PIT tags can be implanted in smaller-bodied fishes and fine-scale movements of individuals can be studied. Moreover, one tag costs less than US$7, making it possible to mark a large number of individuals in the wild. Therefore, the method can be applied to address numerous questions related to animal behaviour and habitat, from fine-scale movements of individuals in the channel to spatial distribution of a local population. The data can also be interfaced with habitat measurements to describe habitat use by individuals and by a species in general. In the case of species that live in deeper habitats, waterproof reading units can be designed for snorkeling or for scuba diving experiments. Unlike radio transmitters, PIT tags do not require batteries to operate. An operational life of 10 years or more is expected and the method, therefore, could be useful for linking the life history of individuals to the dynamics of a population, especially for salmonids. Lastly, the applications of the new tracking method need not be restricted to fish but can be used for many animals with restricted movements in their habitat.

Acknowledgments

Financial support was provided by Le Conseil Régional de Bretagne to J-M.R. (postdoctoral fellowship) and by a Natural Sciences and Engineering Research Council grant to R.A.C. Comments by H. Keckeis and two anonymous reviewers enhanced the final draft. The authors are especially grateful to S. McCormick, R. O'Keefe, P. Hardie, I. Benwell, and T. Matchim for laboratory and field assistance.

References

Adams, N.S, Rondorf, D.W., Evans, S.D., Kelly, J.E., and Perry, R.W. 1998. Effects of surgically and gastrically implanted radio transmitters on swimming performance and predator avoidance of juvenile chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **55**: 781–787.

Armstrong, J.D., Braithwaite, V.A., and Rycroft, P. 1996. A flat-bed passive integrated transponder antennea array for monitoring behaviour of Atlantic salmon parr and other fish. J. Fish Biol. **48**: 539–541.

Castro-Santos, T., Haro, A., and Walk, S. 1996. A passive integrated transponder (PIT) tag system for monitoring fishways. Fish. Res. **28:** 253–261.

Hardie, P., Cunjak, R.A., and Komadina-Douthwright, S. 1998. Fish movement in Catamaran Brook, N.B. (1990–1996). Can. Data Rep. Fish. Aquat. Sci. 1038.

Ombredane, D., Baglinière, J-L., and Marchand, F. 1998. The effects of Passive Integrated Transponder tags on the survival and growth of juvenile brown trout (*Salmo trutta* L.) and their use for studying movement in a small river. Hydrobiologia, **371/372**: 99–106.

Prentice, E.F., Flagg, T.A., and McCutcheon, C.S. 1990. Feasibility of using implantable Passive Integrated Transponder (PIT) tags in salmonids. Am. Fish. Soc. Symp. **7**: 317–322.

Summerfelt, R.C., and Smith, L.S. 1990. Anesthesia, surgery, and related techniques. *In* Methods for fish biology. *Edited by* C.B. Schreck and P.B. Moyle. American Fisheries Society, Bethesda, Md. pp. 213–263.

Bioavailability of sediment-bound metals for *Vallisneria americana* Michx, a submerged aquatic plant, in the St. Lawrence River

Louise St-Cyr and Peter G.C. Campbell

Abstract: We have assessed the bioavailability of sediment-bound metals (Cd, Cr, Cu, Ni, Pb, and Zn) in the St. Lawrence River using a rooted aquatic plant, *Vallisneria americana* Michx, as the biomonitor species. The host sediments were subjected to a sequential leaching procedure to determine the partitioning of the metals present in the surficial oxic stratum; previously published equations were then used to estimate the free metal ion concentrations at the root–sediment interface. Suspected metal accumulation in foliage from point-source pollution of the water column obscured sediment–plant relationships at several sampling stations, but in general, plant metal concentrations could be predicted on the basis of sediment geochemistry (including sediment metal concentrations). Metal concentrations in *Vallisneria* tissues correlated more closely with estimates of bioavailable sediment-bound metal than with total metal concentrations in sediments, particularly for Cd, Pb, and Zn. Roots proved to be better bioindicator organs than shoots for monitoring sediment contamination. Overall, the results demonstrate that *Vallisneria* would be a useful biomonitor species of metal contamination in the St. Lawrence River.

Résumé : Nous avons étudié la biodisponibilité des métaux (Cd, Cr, Cu, Ni, Pb et Zn) dans les sédiments du fleuve Saint-Laurent en utilisant une plante aquatique enracinée submergée, *Vallisneria americana* Michx, comme espèce bioindicatrice. La partie superficielle oxygénée des sédiments a été soumis à une procédure d'extraction séquentielle pour déterminer la spéciation des métaux; des équations déjà publiées ont été utilisées pour estimer les concentrations d'ions libres des métaux à l'interface racines–sédiment. L'accumulation présumée de métaux dans les feuilles à des points géographiques précis où des sources ponctuelles de métaux contaminent la colonne d'eau obscurcit les relations avec les métaux dans les sédiments à quelques stations d'échantillonnage, mais en général, les concentrations de métaux dans les tissus de *Vallisneria* reflètent la partie biodisponible des métaux dans les sédiments, plus que les concentrations totales, particulièrement pour le Cd, le Pb et le Zn. Les racines sont de meilleurs organes bioindicateurs de la contamination dans les sédiments que ne le sont les parties vertes. L'ensemble des résultats montrent que *Vallisneria* pourrait être utilisée comme espèce bioindicatrice de la contamination en métaux dans le fleuve Saint-Laurent.

Introduction

Rooted submerged aquatic plants can potentially absorb metals both through their leaves via the surrounding water (e.g., Welsh and Denny 1980) and through their roots via the sediment interstitial water. Because sediments have a greater potential pool of metals than the overlying water column (e.g., St-Cyr et al. 1994), several researchers have proposed that rooted submerged plants having well-developed root systems and submerged leaves extract metals mostly from the sediment, with subsequent translocation to the upper parts, and have attempted to relate metal levels in sediments to metal concentrations in plant tissues (e.g., Campbell et al. 1985; Jackson 1998).

In looking for sediment–plant relationships, many investigators have worked with total metal concentrations in sediments. However, total metal levels in sediments may in fact not accurately reflect the metal's availability to aquatic plants because a large portion of the measured metal may be present in forms that are refractory, nonlabile, and hence unavailable (Luoma 1989). Jackson et al. (1991), working with data from a broad literature review, reported a significant positive relationship between metal concentrations in the above-ground tissues of aquatic plants (of all growth forms: emergent, submerged, with floating leaves, and free-floating plants) and the total metal concentrations in the underlying sediments but reported that both organic and Fe content (Jackson et al. 1991) as well as sediment pH and redox potentials (Jackson et al. 1993) contribute to ameliorate this relationship.

Given the inability of plants to take up particulate metals directly (the metal must first pass into solution before it can be taken up), a more logical approach would be to consider metal concentrations in sediment interstitial water. However, metal concentrations in interstitial water are normally very low, sample volumes are small, and separation artifacts abound (Adams 1994). The use of partial sediment extractions and geochemical models to estimate porewater metal

Received July 27, 1999. Accepted November 4, 1999.
J15265

L. St-Cyr[1] and P.G.C. Campbell. INRS-Eau, Université du Québec, 2700, rue Einstein, C.P. 7500, Sainte-Foy, QC G1V 4C7, Canada.

[1] Author to whom all correspondence should be sent at the following address: 330 Bienville, Longueuil, QC J4H 2E5, Canada. e-mail: couillay@sympatico.ca

concentrations has been suggested as a means of circumventing these methodological problems (Campbell and Lewis 1988; St-Cyr and Campbell 1996).

In the St. Lawrence River, *Vallisneria americana* Michx (hereafter referred to as *Vallisneria*) has been proposed as a promising biomonitor species for metal contamination: it grows in most submerged plant communities and in all water types and concentrates metals (other than Pb) to higher levels than do the other rooted submerged species analysed (St-Cyr and Campbell 1994). However, for biomonitor species, predictable relationships should exist between the bioaccumulated metal in the plant tissues and the levels of bioavailable metals in the surrounding environment, and these relationships should be robust (i.e., consistent over time (interannual variation) and space (in different study areas)). In an earlier publication, we reported the spatial variability of metal concentrations in *Vallisneria* in the St. Lawrence River (St-Cyr and Campbell 1994). In the present paper, we explore relationships between the metal content of this submerged plant and the potentially bioavailable metal concentrations in the sediments.

Materials and methods

Sampling

Vallisneria is a completely submerged plant, rooted in the sediment, with rosette-type ribbon-like leaves and a tiny stem; leaves can reach a length of nearly 2 m. In August 1990, *Vallisneria* was collected in Lake St. Louis (eight sampling stations) and Lake St. Pierre (nine sampling stations). In August 1991, plants were collected in these two lakes plus Lake St. François and Lake des Deux Montagnes (total of 13 sampling stations). Lakes St. Louis, St. Pierre, and St. François are fluvial lakes of the St. Lawrence River system, while Lake des Deux Montagnes corresponds to a widening of the Ottawa River, an important tributary, just upstream of its confluence with the St. Lawrence River (Quebec, Canada) (Fig. 1). The waters from the Ottawa River are poorly mineralized, with a low alkalinity and relatively high concentrations of fulvic–humic acids, whereas the waters of the St. Lawrence River are more highly mineralized and are richer in carbonates (St-Cyr and Campbell 1994).

At each sampling station, *Vallisneria* rosettes (above- and belowground parts) were carefully disengaged from the sediments by SCUBA divers, put in plastic bags filled with lake water, and placed in a cooler. In the laboratory, plants were carefully washed with tap water, rinsed with deionized water, and either processed immediately for metal analysis (1990 sampling) or frozen until analysis (1991 sampling).

The divers also collected surficial bottom sediments at each sampling station, near the plants to be collected for metal analysis, using a polypropylene corer. In the boat, the core was carefully extruded and the upper layer (about the first 1 cm) was removed using a Teflon sheet and placed in a 500-mL polypropylene bottle previously soaked with acid and rinsed with deionized water. Only the oxic sediments (at the water–sediment interface) were analysed. A composite sample from four cores was prepared at each sampling station to obtain an amount of sediment sufficient for analysis. Lake water was added to the bottles to suspend the sediments and help maintain them in an oxic state. The bottles were placed in a cooler and transported to the laboratory where they were frozen until analysis.

Sediment analysis

All labware was soaked in 15% HNO_3 for 24 h and thoroughly rinsed with deionized water prior to use. Thawed sediments were analysed using the sequential extraction procedure of Tessier et al. (1979) designed to partition metals into operationally defined fractions as follows:

F1 – exchangeable metals. The sediment sample was extracted for 10 min with 0.5 M $MgCl_2$ at pH 7.0.

F2 – metals bound to carbonates or specifically adsorbed. The residue from F1 was leached for 5 h with 1.0 M NaOAc adjusted to pH 5.0 with HOAc.

F3 – metals bound to reducible iron and manganese oxides. The residue from F2 was extracted for 6 h at 96°C with 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc.

F4 – metals bound to organic matter and sulfides. The residue from F3 was extracted at 85°C for 5 h with 30% H_2O_2 adjusted to pH 2.0 with HNO_3 and then at room temperature with 3.2 M NH_4OAc in 20 % (v/v) HNO_3.

F5 – residual metals. The residue from F4 was extracted using the same procedure as for total metal determination.

The sum of the first three fractions (i.e., M(S3) = F1 + F2 + F3) is presumed to represent the potentially available sediment-bound metal concentrations. A second subsample was analysed for total metals after digestion with a 5:1 mixture of HF and $HClO_4$. Sediment samples certified for total metal concentrations (MEIS-I; National Research Council of Canada, Ottawa, Ont.) were analysed in parallel. For these samples, the sum of metal concentrations in the five fractions of the sequential extraction procedure ($\Sigma F1 \rightarrow F5$) ($N = 2$) did not differ significantly ($P > 0.05$) from the concentration of the direct total metal extraction ($N = 3$) as compared using Student's *t* test comparison of means for small samples for each metal studied. These concentration values fell within or close to the expected certified range for each metal. Metal concentrations in the leachates were measured using either a flame atomic absorption spectrophotometer (AAS), a graphite furnace AAS, or an inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Calibration curves were obtained using as an analytical matrix solutions of the same composition as those used for each extraction.

Sediment organic C concentrations were determined with a CNS analyser after removal of inorganic C by acidification with H_2SO_4. The pH of the wet sediment was measured in 0.01 M $CaCl_2$ (Peech 1965) using a 1:1 ratio of extractant – equivalent dry weight sediments.

Dissolved free Cd^{2+} concentrations at the root–sediment interface were estimated on the basis of the sorption of this metal to sedimentary organic matter and iron oxyhydroxides. The rationale for the following equation has been developed in Tessier et al. (1993):

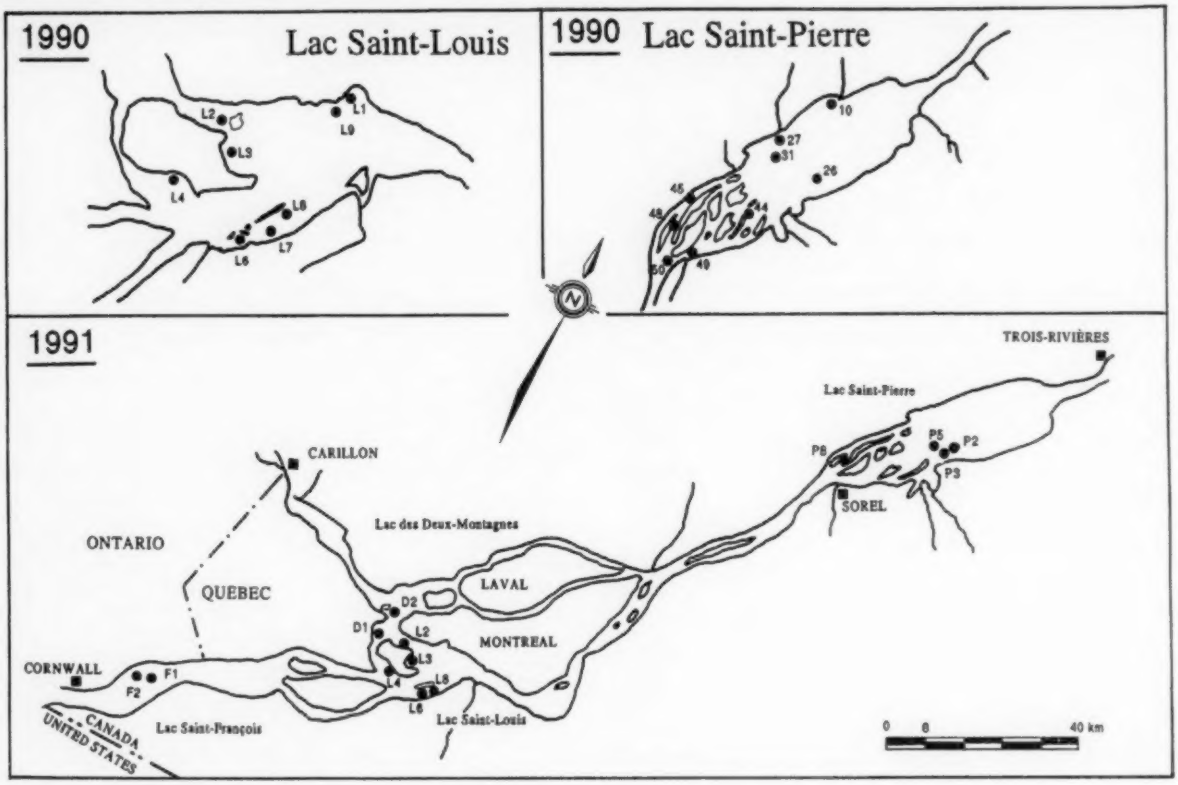
$$[Cd^{2+}](mol \cdot L^{-1}) = \frac{\{Cd_S\}[H^+]^{1.79}}{10^{-1.30}\{Fe\text{-}ox\}[H^+]^{0.97} + 10^{-2.45}\{OM\}[H^+]^{0.82}}$$

where $\{Cd_S\}$ is the sum of the nondetrital Cd concentrations (moles per gram) as found in the four fractions extracted from the sediment, $\{Fe\text{-}ox\}$ is the Fe concentration (moles per gram) in F3 extracted from the sediment, and $\{OM\}$ is the sediment organic C (moles per gram). The basis for the equation used to estimate dissolved Pb concentrations at the root–sediment interface can be found in Tessier (1992):

$$[Pb^{2+}](mol \cdot L^{-1}) = \frac{\{Pb(S3)\}[H^+]^{0.81}}{10^{0.67}\{Fe(F3)\}}$$

where $\{Pb(S3)\}$ is the sum of Pb concentrations (moles per gram) as found in the three first fractions extracted from the sediment (F1 + F2 + F3) and $\{Fe(F3)\}$ is the Fe concentrations (moles per gram) in F3 extracted from the sediment. Values for $[Cu^{2+}]$, $[Ni^{2+}]$, and $[Zn^{2+}]$ were also determined as outlined in Tessier (1992). There is no published equation to estimate $[Cr^{3+}]$. Note that the equation used to estimate $[Cd^{2+}]$ has been refined and tested over a wide geographical area (Tessier et al. 1993), whereas the geochem-

Fig. 1. Map of the sampling stations in the St. Lawrence River fluvial lakes.



ical models used to calculate [Cu^{2+}],[Ni^{2+}], [Pb^{2+}], and [Zn^{2+}] are less well tested (Tessier 1992).

Plant analysis

Two to four composite samples (of three to five rosettes) were prepared for each sampling station. Plant above- and below-ground parts were separated. Roots were first treated to remove the iron oxide coating surrounding them using the DCB (sodium dithionite – sodium citrate – sodium bicarbonate) method (Taylor and Crowder 1983; Otte et al. 1989). The dithionite product used for analysis of roots collected in 1990 was found to be highly contaminated with Zn; however, in 1991, we changed our supplier and the contamination from the dithionite reagent used was then negligible (St-Cyr and Campbell 1996). Consequently, Zn values for root tissues are only available for the 1991 collections.

Cleaned roots and leaves were dried at 60°C for 24 h, ground to a powder with liquid nitrogen, and then digested (0.1–0.5 g) with concentrated HNO_3 (Aristar grade) in Teflon bombs in a microwave oven. Sample digests were analysed for Cd, Cu, Cr, Ni, and Zn using ICP-AES. Lead concentrations were measured using AAS. Samples collected during the August 1990 sampling yielding values of Cd, Cr, Ni, or Pb below the detection limits of these two analytical methods were reassayed using graphite furnace AAS. For the 1991 tissues, only the Cd values were reassayed in this manner with the more sensitive technique. Certified reference material from the U.S. National Institute of Standards and Technology (citrus leaves SRM 1572) was digested during each analytical run. Measured metal concentrations compared well with the certified values for Cu, Zn, and Pb. For Cd, Cr, and Ni, the certified values fell below the detection limit of the analytical method.

Statistical analysis

Initially, relationships were examined between metals in sediments and in roots, where metal uptake is thought primarily to occur. Only the oxic sediments (at the water–sediment interface) were analysed, as they were thought to better reflect the oxygenated sediments surrounding the roots in the rhizosphere (Good and Patrick 1987). We looked first for relationships with the total metal concentrations in the sediments and then with the calculated free metal ion concentration in the sediment interstitial water, [M^{Z+}]$_i$, and the readily extractable metal concentration, [M(S3)]. Subsequently, relationships between metals in roots and above-ground parts (leaves) of the plant were examined, and finally, we looked for relationships between metals in the leaves and metal concentrations in the sediments.

Pearson's correlation tests were used to identify potential relationships between variables; unless otherwise indicated, a threshold value of $P = 0.05$ was used to test for the statistical significance of the relationships. If the assumption of normality required for use of parametric statistical analysis was not met, data were log transformed. The Hotelling distribution of T^2 was used to exclude outlier data (Jolicoeur 1997).

Results

Ranges and means of Cd, Cr, Cu, Ni, Pb, and Zn concentrations determined in *Vallisneria* leaves and roots collected in the St. Lawrence River are listed in Table 1 as well as the ranges and means of total metal concentrations in the sediments underlying these plants. Cadmium and Pb concentrations were generally higher in *Vallisneria* root tissues than in the leaves; the same was true for Cu and Cr but with more variability. Zinc and Ni concentrations, however, were higher in the leaves than in the roots. The complete listing of metal concentrations in plant tissues and metal partitioning in the sediments, expressed as [M(S3)], total [M], and $[\mathrm{M}^{Z+}]$ at each sampling station, can be found in Table 2; pH values, [Fe(F3)], and the organic C content of the sediments at each station are reported in Table 3.

Plant versus total sediment metal concentrations

Concentrations of Cd, Pb, Cu, and Ni in *Vallisneria* roots were not significantly related to these same total metal concentrations in the underlying sediments (Figs. 2*a* and 3*a*), but significant relationships were obtained for Zn (Fig. 4*a*) and Cr ($N = 17$, $r = 0.526$, $P < 0.05$). In the case of foliage, only leaf Zn concentrations were significantly related to the corresponding total metal in the sediments ($N = 13$, $r = 0.622$, $P < 0.05$). Indeed, in the case of Cu, analysing relationships between [Cu] in *Vallisneria* leaves and the total Cu concentrations in sediments from two different lakes gave diametrically different results; data were positively related in one lake, whereas a negative correlation was obtained in the other lake (Figs. 5*b* and 5*c*).

Plant versus calculated free metal ion concentrations

The calculated values of $[\mathrm{Cd}^{2+}]$ and $[\mathrm{Pb}^{2+}]$ related well with the Cd and Pb concentrations in roots of *Vallisneria* (Figs. 2*b* and 3*b*). Note, for example, that sampling stations 26 and L2, which had the highest total [Pb] in the sediments, showed relatively low concentrations of estimated Pb^{2+} (Fig. 3) and correspondingly low values for root Pb concentrations. In the case of Cd (Fig. 2*b*), data from stations L1 and D1 were excluded from the correlation, being different from the statistical population (using the Hotelling distribution of T^2, D1 at $P < 0.001$ and L1 at $P < 0.01$). At station D1, [Cd] in the leaves was much higher than in the roots, in contrast with the other stations; [Cd] in the roots was much lower than expected. At site L1, *Vallisneria* were rooted in a rough substrate, with roots extending through rocks and pebbles; the superficial sediments collected between these pebbles perhaps do not accurately reflect the geochemical variables of the rooting medium (pH, organic matter content) extending below the rocky surface, leading to a calculated $[\mathrm{Cd}^{2+}]$ that underestimates the ambient exposure conditions.

Positive relationships were obtained between concentrations in above- and below-ground parts for Cd ($N = 30$, $r = 0.614$, $P < 0.001$) and Pb ($N = 17$, $r = 0.537$, $P < 0.05$). A significant correlation was obtained between [Cd] in leaves and $[\mathrm{Cd}^{2+}]$ ($N = 30$, $r = 0.598$, $P < 0.001$) but not for [Pb].

Plant versus extractable metal concentrations

No significant relationships were obtained relating [Zn], [Cu], and [Ni] in *Vallisneria* tissues with values of $[\mathrm{Zn}^{2+}]$, $[\mathrm{Cu}^{2+}]$, and $[\mathrm{Ni}^{2+}]$ calculated for the sediment interstitial water. In the case of Zn and Cu, normalizing the readily extractable metal concentrations in the sediments, [M(S3)], with respect to the iron oxyhydroxide content, [Fe(F3)], improves the relationship between these metals in *Vallisneria* roots and in sediments (Fig. 4*b*) (for Cu, $N = 30$, $r = 0.396$, $P < 0.05$). Positive relationships were obtained between concentrations in above- and below-ground parts for Zn ($N = 12$, $r = 0.697$, $P < 0.05$) and Cu ($N = 30$, $r = 0.509$, $P < 0.01$).

Table 1. Range of metal concentrations in *V. americana* roots and leaves and range of total metal concentrations in sediments (mean in parentheses) for all stations and sampling dates combined for the study of the St. Lawrence River fluvial lakes.

Metal	[M] in roots ($\mu g \cdot g^{-1}$)	[M] in leaves ($\mu g \cdot g^{-1}$)	[M] total in sediments ($\mu g \cdot g^{-1}$)
Cd	0.28–4.3 (1.6)	0.07–2.35 (0.93)	0.22–3.15 (1.0)
Pb	3.2–20.2 (9.8)	0.6–10.9 (3.0)	17.6–56.2 (29.5)
Cu	4.4–39.4 (20.7)	6.2–34.5 (15.7)	5.7–134 (49.8)
Zn	6.1–246 (41)	39.9–351 (108)	59.2–552 (194)
Ni	0.4–3.5 (1.9)	2.7–28.1 (7.9)	20.4–117 (44)
Cr	2.1–9.5 (3.6)	0.9–7.2 (2.8)	53.6–266 (101)

The most readily extractable forms of Ni and Cr in the sediments ([Ni(S3] and [Cr(S3)]) showed significant relationships with these metal concentrations in *Vallisneria* roots (for Ni, $N = 17$, $r = 0.548$, $P < 0.05$; for Cr, $N = 17$, $r = 0.531$, $P < 0.05$). Both metals show much higher above-ground tissue concentrations at station 49 in Lake St. Pierre (Table 2), and both metals fail to show any significant correlations between shoot and root or sediment ([M(S3)]) metal concentrations.

Discussion

Metal uptake by rooted submerged plants

For submerged aquatic plants, it is now generally accepted that the major route of uptake of nutrients, such as P and N, is through the root system; however, the relative proportion of uptake by the roots and shoots depends on the water to sediment available nutrient ratio (Rattray et al. 1991). For metals, theoretically, both routes could also be used. For species such as *Vallisneria*, having well-developed root systems and totally submerged foliage and considering the generally very low concentrations of metals in the overlying water compared with the high potential pool retained in the sediment (St-Cyr et al. 1994), one might expect the plant to extract metals mostly from the sediments via the root hairs, with subsequent translocation to the upper parts, acropetal transport of water and inorganic elements being a recognized phenomenon in submerged plants (Pedersen and Sand-Jensen 1993). However, uptake by submerged foliage would be expected to become important when metal concentrations in the surrounding water are high and (or) when metal concentrations in the sediment interstitial water are low (Guilizzoni 1991).

Iron oxyhydroxide accumulation is often noted on the roots of aquatic plants, including *Vallisneria*. These Fe "plaques" can bind nutrients and other metals; if not properly removed, the iron coatings and their accompanying metals can swamp the true metal content of the roots (St-Cyr and Campbell 1996). The DCB cleanup procedure used in the present study has proved to be effective and relatively gentle for the root tissues (St-Cyr and Campbell 1996). To our knowledge, the present study represents the first time that metals in sediments in the field have been related to the

Table 2. Mean concentrations of potentially available sediment-bound metals, total metal concentrations in sediments, calculated free metal ion concentrations in the interstitial water, and mean (±SD) metal concentrations in roots and leaves of *V. americana* for each station sampled in 1990 and 1991 in the St. Lawrence River fluvial lakes.

Station	Metal	[M(S3)] (µg·g^{-1})	[M] total (µg·g^{-1})	$[M^{Z+}]_i$ (mol·L^{-1})	[M] in roots (µg·g^{-1})	[M] in leaves (µg·g^{-1})
1990						
10	Cd	0.12	0.5	5.70×10^{-11}	0.95 (0.33)	0.58 (0.11)
	Pb	4	20.8	1.13×10^{-10}	3.7 (0.5)	2.8 (0.7)
	Cu	3.2	14.7	1.62×10^{-8}	16.2 (0.2)	9.1 (1.0)
	Zn	33.7	129	1.61×10^{-8}	—	73.2 (13.1)
	Ni	3.5	26.6	8.06×10^{-9}	0.9 (0.2)	2.7 (0.2)
	Cr	5.6	72.8	—	2.3 (0.1)	1.8 (0.2)
26	Cd	0.29	0.59	3.22×10^{-11}	0.75 (0.03)	1.09 (0.26)
	Pb	24.3	56.2	1.89×10^{-10}	11.0 (0.4)	3.2 (0.7)
	Cu	6.1	129	9.54×10^{-9}	39.4 (2.2)	19.1 (2.0)
	Zn	131	264	1.36×10^{-8}	—	351 (15)
	Ni	32.6	117	1.81×10^{-8}	2.3 (0.3)	15.8 (2.9)
	Cr	55.6	266	—	4.7 (0.6)	3.3 (0.2)
27	Cd	0.29	0.62	1.04×10^{-10}	1.36 (1.10)	1.96 (0.36)
	Pb	10	20.6	3.48×10^{-10}	10.4 (3.1)	0.8 (0.4)
	Cu	4	25.4	2.50×10^{-8}	22.0 (2.7)	15.1 (0.9)
	Zn	50.5	133	3.04×10^{-8}	—	127 (12)
	Ni	5.6	32.6	1.62×10^{-8}	1.7 (2.7)	8.1 (1.8)
	Cr	6.9	84.5	—	2.9 (0.6)	1.0 (0.3)
31	Cd	0.19	0.23	1.78×10^{-10}	2.75 (1.04)	1.00 (0.10)
	Pb	6	17.6	3.09×10^{-10}	12.6 (1.4)	1.8 (0.1)
	Cu	3	8.6	2.83×10^{-8}	25.2 (2.4)	9.6 (0.6)
	Zn	25.1	86.6	2.08×10^{-8}	—	92.0 (16.0)
	Ni	4.1	22.5	1.69×10^{-8}	1.0 (0.2)	3.0 (0.5)
	Cr	4	71.9	—	2.3 (0.1)	0.9 (0)
44	Cd	0.55	1.08	5.47×10^{-11}	1.25 (0.16)	0.41 (0.01)
	Pb	12.4	25.8	1.84×10^{-10}	8.6 (1.2)	1.6 (0.2)
	Cu	0.8	39.3	2.31×10^{-9}	13.8 (1.3)	9.5 (1.9)
	Zn	62	179	1.18×10^{-8}	—	124 (33)
	Ni	8.6	40.7	8.83×10^{-9}	0.4 (0.2)	4.7 (0.4)
	Cr	5.9	98.4	—	2.1 (0)	3.1 (0.1)
45	Cd	0.39	0.74	1.96×10^{-10}	0.56 (0.17)	0.60 (0.12)
	Pb	8.1	23.2	4.12×10^{-10}	7.9 (2.6)	7.4 (1.8)
	Cu	2.4	38.1	1.99×10^{-8}	11.5 (3.7)	12.5 (0.5)
	Zn	49	169	5.59×10^{-8}	—	112 (12)
	Ni	5.7	30.3	2.78×10^{-8}	1.9 (0.5)	4.3 (0.5)
	Cr	4.4	79.3	—	3.7 (1.2)	4.5 (0.6)
48	Cd	0.95	2.12	3.96×10^{-10}	1.40 (0.23)	2.06 (0.39)
	Pb	22.2	37.7	6.91×10^{-10}	20.2 (2.7)	5.5 (1.2)
	Cu	7.6	82.9	3.78×10^{-8}	30.0 (3.2)	21.5 (0.6)
	Zn	116	324	8.43×10^{-8}	—	135 (17)
	Ni	12.5	54	3.83×10^{-8}	2.6 (0.1)	9.5 (0.9)
	Cr	15.2	121	—	4.8 (1.1)	2.4 (0.3)
49	Cd	0.09	0.43	5.07×10^{-11}	1.12 (0.36)	0.54 (0.10)
	Pb	10.1	23.8	3.29×10^{-10}	18.0 (3.0)	10.9 (2.0)
	Cu	2.2	13.6	1.44×10^{-8}	36.5 (9.1)	22.3 (2.5)
	Zn	19.5	79.4	8.69×10^{-9}	—	154 (30)
	Ni	4.5	33.2	1.06×10^{-8}	2.2 (0.8)	28.1 (3.5)
	Cr	7	95.8	—	4.0 (1.2)	7.2 (4.1)
50	Cd	0.61	1.14	1.91×10^{-10}	1.04 (0.30)	1.49 (0.22)
	Pb	19.5	35.4	3.56×10^{-10}	10.7 (2.6)	3.4 (1.3)
	Cu	5.6	64.4	1.93×10^{-8}	30.8 (6.8)	18.0 (2.7)
	Zn	85	255	2.43×10^{-8}	—	130 (33)
	Ni	7.6	38.7	1.09×10^{-8}	2.9 (0.5)	12.1 (3.1)
	Cr	9.7	92	—	5.6 (1.7)	2.6 (1.2)

Table 2 (*continued*).

Station	Metal	[M(S3)] (µg·g^{-1})	[M] total (µg·g^{-1})	[M^{Z+}]$_i$ (mol·L^{-1})	[M] in roots (µg·g^{-1})	[M] in leaves (µg·g^{-1})
L2	Cd	1.44	2.45	1.16×10^{-10}	0.81 (0.10)	0.43 (0.25)
	Pb	20.2	50.4	2.13×10^{-10}	5.3 (1.1)	1.0 (0.5)
	Cu	1.1	46.7	2.13×10^{-9}	12.4 (0.3)	12.9 (1.1)
	Zn	168	362	2.75×10^{-8}	—	93.8 (32.5)
	Ni	13.4	57.2	1.11×10^{-8}	3.5 (1.2)	4.1 (0.3)
	Cr	9.5	121	—	9.5 (2.9)	1.2 (0.1)
L3	Cd	1.12	2.07	1.27×10^{-10}	2.26 (0.40)	0.67 (0.43)
	Pb	14.9	32.1	2.11×10^{-10}	3.2 (0.7)	1.2 (0.3)
	Cu	0.6	41.4	1.76×10^{-9}	17.7 (3.0)	15.0 (0.6)
	Zn	127	270	2.57×10^{-8}	—	80.4 (11.1)
	Ni	8.9	41.6	9.42×10^{-9}	2.1 (1.8)	4.4 (0.7)
	Cr	6.9	98.3	—	2.1 (0.1)	1.5 (0.1)
L4	Cd	0.51	0.7	2.26×10^{-10}	4.30 (1.16)	1.60 (0.52)
	Pb	14.8	26.9	5.12×10^{-10}	15.8 (0.5)	3.6 (0.3)
	Cu	1.8	21	1.04×10^{-8}	33.6 (4.3)	22.8 (0.6)
	Zn	72.9	176	5.53×10^{-8}	—	106 (3.5)
	Ni	5.2	38.5	1.69×10^{-8}	1.4 (0.4)	6.3 (0.6)
	Cr	5.8	82	—	3.3 (0.6)	1.0 (0)
L6	Cd	0.67	0.71	5.82×10^{-11}	0.64 (0.07)	0.27 (0.11)
	Pb	12.9	17.6	2.58×10^{-10}	6.1 (0.2)	0.6 (0)
	Cu	0.4	27.6	1.52×10^{-9}	9.9 (0.2)	6.2 (0.6)
	Zn	56.9	133	1.59×10^{-8}	—	39.9 (5.5)
	Ni	7.1	33.5	1.04×10^{-8}	2.2 (0.4)	5.7 (0.3)
	Cr	5.4	72.9	—	2.2 (0.3)	1.3 (0.3)
L7	Cd	0.93	1.4	6.10×10^{-11}	1.60 (0.89)	0.74 (0.10)
	Pb	24.3	41.2	4.67×10^{-10}	8.7 (5.2)	1.1 (0.4)
	Cu	0.6	48.9	2.09×10^{-9}	18.7 (11.9)	8.9 (6.5)
	Zn	90.1	222	2.53×10^{-8}	—	116 (28)
	Ni	11.3	54.1	1.64×10^{-8}	3.2 (1.2)	9.1 (0.9)
	Cr	7.7	113	—	3.3 (1.1)	6.3 (0.5)
L8	Cd	0.78	1.39	4.05×10^{-11}	1.34 (0.27)	0.43 (0.08)
	Pb	16.1	30.3	2.98×10^{-10}	7.4 (0.1)	0.9 (0.3)
	Cu	0.5	43.6	1.68×10^{-9}	36.5 (12.4)	13.0 (2.0)
	Zn	84.2	193	2.08×10^{-8}	—	70.8 (6.1)
	Ni	12.1	47.3	1.59×10^{-8}	3.0 (0.9)	6.2 (1.6)
	Cr	6.9	88.6	—	3.2 (0.4)	4.2 (2.9)
L9	Cd	0.21	0.37	1.77×10^{-10}	2.69 (0.04)	1.11 (0.15)
	Pb	6.1	18	3.32×10^{-10}	9.9 (1.6)	3.4 (0.2)
	Cu	1.5	5.7	1.50×10^{-8}	36.0 (1.9)	28.6 (3.7)
	Zn	22.8	70.9	2.12×10^{-8}	—	75.8 (1.2)
	Ni	2.6	20.4	1.15×10^{-8}	0.5 (0.5)	4.9 (1.1)
	Cr	2.5	53.6	—	2.3 (0.2)	3.2 (2.8)
L1	Cd	0.52	3.15	6.02×10^{-11}	4.26 (0.67)	1.87 (0.12)
	Pb	6.1	24.4	6.86×10^{-11}	6.9 (1.1)	1.5 (0.2)
	Cu	1.5	16.3	3.41×10^{-9}	28.3 (2.2)	24.2 (0.8)
	Zn	94.5	313	1.47×10^{-8}	—	144 (45)
	Ni	8	61	6.56×10^{-9}	0.9 (1.5)	4.8 (0.3)
	Cr	4.8	107	—	3.3 (1.8)	1.5 (0.1)
1991	Cd	0.92	0.7	1.05×10^{-9}	3.77 (0.13)	2.35 (0.24)
L2	Cu	1.9	54.6	1.99×10^{-8}	22.6 (0.1)	12.3 (3.3)
	Zn	94.1	132	3.34×10^{-7}	8.7 (0.1)	55.1 (11.4)
L3	Cd	1.51	0.9	4.49×10^{-10}	3.11 (0.88)	0.84 (0.06)
	Cu	1.5	27.9	7.22×10^{-9}	16.0 (4.3)	9.9 (3.7)
	Zn	116	139	4.66×10^{-8}	49.8 (0.2)	94.7 (17.7)
L4	Cd	0.25	0.38	1.82×10^{-10}	1.3	1.01
	Cu	1.6	36.4	7.82×10^{-9}	9.8	16.9
	Zn	18.1	59.2	6.86×10^{-9}	8.7	49

Table 2 (*concluded*).

Station	Metal	[M(S3)] (μg·g^{-1})	[M] total (μg·g^{-1})	$[M^{Z+}]_i$ (mol·L^{-1})	[M] in roots (μg·g^{-1})	[M] in leaves (μg·g^{-1})
L6	Cd	0.82	0.6	4.96×10^{-11}	1.17 (0.68)	0.30 (0.06)
	Cu	1.1	46.1	3.13×10^{-9}	9.0 (3.2)	7.9 (1.4)
	Zn	59.4	78.1	9.27×10^{-9}	14.2 (8.0)	61.6 (19.3)
L8	Cd	0.61	0.61	7.25×10^{-11}	2.79 (0.03)	0.80 (0.06)
	Cu	1.3	33.7	5.28×10^{-9}	12.1 (2.2)	7.7 (1.1)
	Zn	48	93.8	8.95×10^{-9}	27.2 (2.1)	89.0 (2.8)
F1	Cd	0.85	1.38	5.31×10^{-11}	0.28 (0.07)	0.07 (0.03)
	Cu	0.8	86.5	3.48×10^{-9}	13.9 (8.8)	13.6 (11.5)
	Zn	418	552	1.44×10^{-7}	57.7 (16.6)	95.8 (11.8)
F2	Cd	0.66	0.83	4.17×10^{-11}	0.45 (0.10)	0.12 (0.03)
	Cu	0.3	61.1	9.25×10^{-10}	18.0 (8.8)	14.9 (6.7)
	Zn	209	340	4.53×10^{-8}	56.5 (1.9)	206 (37)
P2	Cd	0.19	0.69	5.38×10^{-11}	0.67 (0.88)	0.53 (0.06)
	Cu	3.8	131	1.02×10^{-8}	13.3 (10.0)	34.5 (26.7)
	Zn	90.5	185	2.11×10^{-8}	15.2 (4.7)	116 (29)
P3	Cd	0.3	0.56	1.69×10^{-10}	1.17 (0.01)	0.58 (0.02)
	Cu	2.4	113	8.06×10^{-9}	21.3 (1.8)	16.8 (2.1)
	Zn	77.2	169	2.37×10^{-8}	13.3 (9.1)	96.7 (4.9)
P5	Cd	0.1	0.22	3.26×10^{-11}	0.61 (0.24)	0.49 (0.13)
	Cu	2.5	73.2	1.05×10^{-8}	23.9 (9.5)	19.6 (17.0)
	Zn	33.3	105	9.35×10^{-9}	14.9 (2.0)	102 (47)
P8	Cd	1.07	1.29	4.85×10^{-10}	0.67	0.85
	Cu	2.8	83.6	2.43×10^{-8}	20.4	19.7
	Zn	103	242	1.46×10^{-7}	246	92.8
D1	Cd	0.63	1.21	1.28×10^{-9}	0.42	1.7
	Cu	2.6	57.1	2.60×10^{-8}	4.4	12.1
	Zn	98.2	224	4.29×10^{-7}	15.4	80.1
D2	Cd	0.1	0.82	7.05×10^{-10}	2.57	1.43
	Cu	0.7	22.7	2.41×10^{-8}	16.9	17.6
	Zn	14.1	135	9.56×10^{-8}	6.1	65.5

true metal content of plant roots, cleaned from external contamination.

Relationships between [M] in plant and total [M] in sediment

Vallisneria root Zn and Cr concentrations were significantly related to these same total metal concentrations in the underlying sediments, but no relationships were obtained for the other metals studied (Cd, Cu, Pb, and Ni). This inability to predict for all metals studied the plant metal levels (particularly in the leaves) on the basis of total metal concentrations in the sediments contrasts with the results previously obtained by Jackson et al. (1991), where total metal concentrations in sediments were reported to be a good predictor of the metal content of aquatic plants. Data leading to their conclusion came from an extensive literature review, covering a very wide metal concentration gradient. For example, in the case of Pb, the sediments analysed contained from 3 to 21 000 μg Pb·g dry weight^{-1}. Over so wide a gradient, one might intuitively expect an empirical relationship to exist between total metal concentrations in sediments and those in plant tissues. However, in practice, aquatic plants are more likely to be used as bioindicators of metals in their environment over a somewhat narrower gradient of contamination. For example, in the St. Lawrence River system covered by our study, total Pb concentrations in sediments varied from 18 to 56 μg Pb·g^{-1}, and no significant relationship was obtained with Pb levels in plant tissues.

It is now generally accepted that total metal concentrations in sediments overestimate the actual bioavailable metal concentrations for benthic organisms, including rooted plants (Campbell and Lewis 1988). Total metal determinations will inevitably include an important fraction of residual metals, held within the lattice of detrital minerals. These metals would not be expected to be released into solution over a reasonable time span under the conditions normally encountered in nature. Given the accumulated evidence that aquatic organisms tend to respond to variation in the free metal ion concentrations (Campbell 1995) and the reported improvement of predictions when variables such as pH, redox potential, and sediment organic content are taken into consideration (Jackson et al. 1993), we have chosen to focus our attention on these sediment variables, considering metal geochemistry in predicting metal uptake by aquatic plants.

Relationships between [M] in plants and surrogate measures of $[M^{Z+}]_i$ at the root–sediment interface

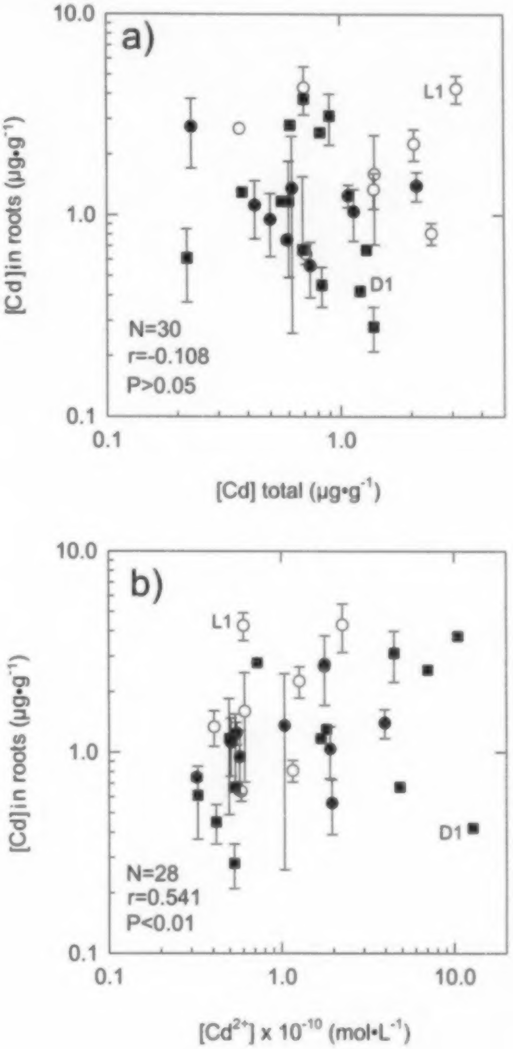
Free metal ion concentrations in the sediment interstitial water would appear to be a more mechanistically meaningful measure of metal availability to rooted aquatic plants, but determinations of $[M^{Z+}]_i$ in interstitial water are notoriously difficult to perform. Direct determination has rarely proved

Table 3. pH, iron oxyhydroxide, and organic C concentrations in the oxic surficial sediments (at the water–sediment interface) at each sampling station in 1990 and 1991 in the St. Lawrence River fluvial lakes.

Station	pH	[Fe(F3)] ($mol \cdot g^{-1}$)	[Organic C] ($mol \cdot g^{-1}$)
1990			
10	6.9	8.9×10^{-5}	8.9×10^{-4}
26	7.2	2.0×10^{-4}	2.2×10^{-3}
27	6.9	7.7×10^{-5}	1.3×10^{-3}
31	7	4.6×10^{-5}	4.1×10^{-4}
44	7.2	9.7×10^{-5}	2.3×10^{-3}
45	6.6	8.6×10^{-5}	1.7×10^{-3}
48	6.6	1.6×10^{-4}	3.0×10^{-3}
49	7.2	4.9×10^{-5}	4.4×10^{-4}
50	7	1.2×10^{-4}	1.8×10^{-3}
L2	7	2.0×10^{-4}	4.5×10^{-3}
L3	7.1	1.2×10^{-4}	2.6×10^{-3}
L4	6.7	1.2×10^{-4}	1.8×10^{-3}
L6	7.1	8.5×10^{-5}	3.3×10^{-3}
L7	7.1	9.7×10^{-5}	5.0×10^{-3}
L8	7.2	8.4×10^{-5}	5.0×10^{-3}
L9	6.9	4.6×10^{-5}	5.1×10^{-4}
L1	7.2	1.4×10^{-4}	2.3×10^{-3}
1991			
L2	6	1.8×10^{-4}	3.3×10^{-3}
L3	7	8.3×10^{-5}	1.2×10^{-3}
L4	7.1	7.3×10^{-5}	3.7×10^{-4}
L6	7.3	8.8×10^{-5}	3.1×10^{-3}
L8	7.5	5.2×10^{-5}	1.2×10^{-3}
F1	7.1	8.9×10^{-5}	5.7×10^{-3}
F2	7.3	8.1×10^{-5}	3.6×10^{-3}
P2	7	1.5×10^{-4}	1.3×10^{-3}
P3	7	1.3×10^{-4}	1.3×10^{-3}
P5	7.2	7.6×10^{-5}	7.5×10^{-4}
P8	6.5	1.0×10^{-4}	3.3×10^{-3}
D1	5.8	2.6×10^{-4}	2.8×10^{-3}
D2	6.4	3.1×10^{-5}	1.9×10^{-4}

feasible, principally due to the relatively low metal concentrations involved and the difficulty in obtaining sufficient sample volumes. For example, the calculated [Cd^{2+}] values in the sediment interstitial water at the stations visited in the St. Lawrence River are very low, ranging from 3.2×10^{-11} to 1.2×10^{-9} $mol \cdot L^{-1}$. However, using partial extraction procedures to partition metals in sediments among various defined forms, one can derive surrogate measures of the free metal ion concentration in the interstitial water, $[M^{Z+}]_i$ (Campbell and Tessier 1996). This approach assumes that the free metal ion concentration at the root–sediment interface is controlled by competitive sorption of the metal on the various sediment phases present in oxic sediments (Tessier 1992).

Fig. 2. Relationships between Cd concentration (±SD) in roots of *V. americana* collected in 1990 (○, Lake St. Louis; ●, Lake St. Pierre) and 1991 (■) versus (*a*) total Cd concentrations in the sediments and (*b*) the estimated free Cd^{2+} concentrations at the root–sediment interface. Note the logarithmic scales. Data from stations L1 and D1 were excluded from the correlation with free Cd^{2+} concentrations, being different from the statistical population (see the text).



The calculated values of [Cd^{2+}] and [Pb^{2+}] from published equations (Tessier 1992; Tessier et al. 1993) related well with the Cd and Pb concentrations in roots of *Vallisneria*. For both metals, greater concentrations were found in the roots than in the leaves of *Vallisneria*, and the metal contents of leaves and roots were significantly correlated. The calculated values of [Cd^{2+}] in the sediment interstitial water were also significantly related to the Cd concentrations in the leaves.

In the case of Zn and Cu, normalizing the readily extractable metal concentrations in the sediments with respect to the iron oxyhydroxide concentrations improves the relationship between these metals in *Vallisneria* roots and in sediments. This role of sediment iron oxyhydroxides in controlling metal availability to various aquatic species has already been reported (Campbell and Tessier 1996). A similar relationship was also obtained when relating Zn concentra-

Fig. 3. Relationships between Pb concentrations (±SD) in roots of *V. americana* collected in 1990 (○, Lake St. Louis; ●, Lake St. Pierre) versus (*a*) total Pb concentrations in the sediments and (*b*) the estimated free Pb^{2+} concentrations at the root–sediment interface.

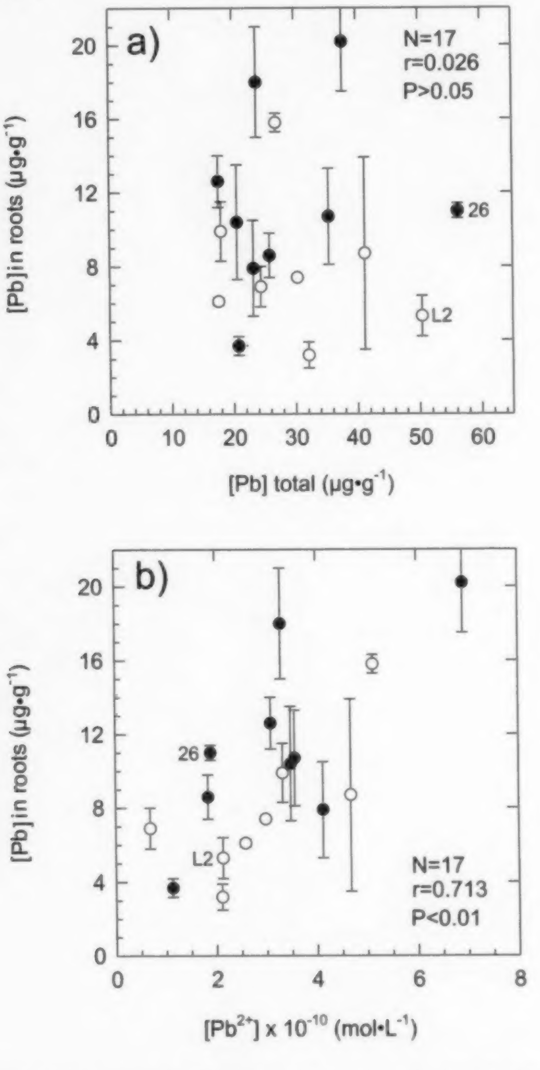
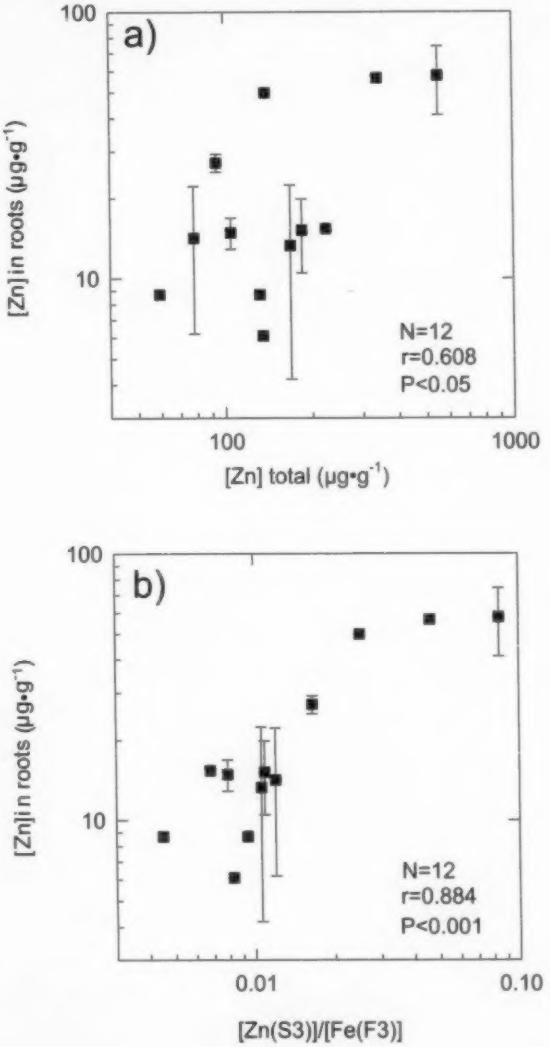


Fig. 4. Relationships between Zn concentrations (±SD) in roots of *V. americana* collected in 1991 versus (*a*) total Zn concentrations in the sediments and (*b*) the [Zn(S3)]/[Fe(F3)] ratio in the sediments. Note the logarithmic scales. Root Zn data from station P8 have been removed from the statistical calculations because Zn concentration in roots at this station (246 $\mu g \cdot g$ dry weight^{-1}) is much more elevated than at the other stations (mean 24 $\mu g \cdot g$ dry weight^{-1}), probably due to inadvertent contamination; this is not observed for leaf Zn concentration from the same station.

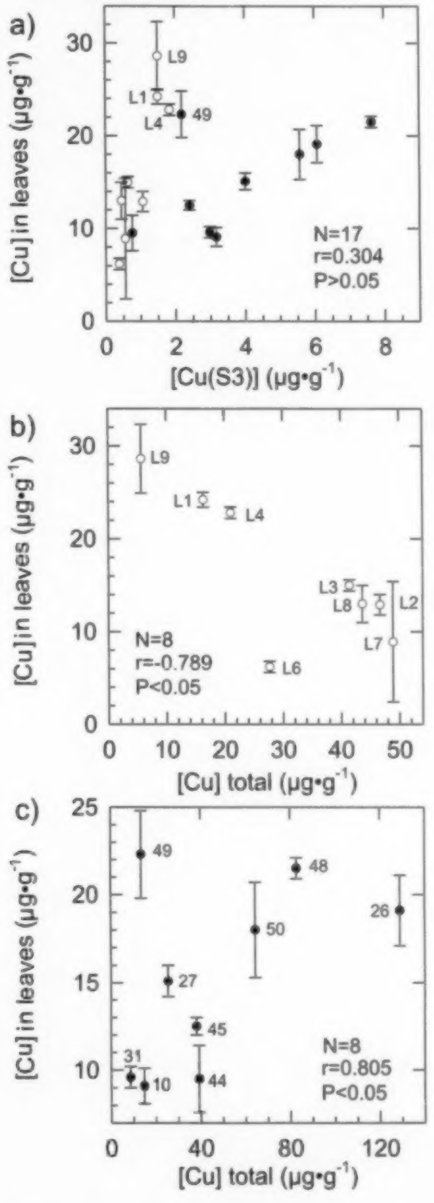


tions in the root Fe plaque of *Vallisneria* with internal Zn root concentrations (St-Cyr and Campbell 1996). There is some geochemical basis for this improved relationship, in that the [Zn]/[Fe(F3)] or [Cu]/[Fe(F3)] ratio can under certain circumstances be considered as a surrogate measure for free Zn^{2+} and Cu^{2+} in the interstitial water surrounding the root (St-Cyr and Campbell 1996).

Role of foliar metal uptake

To this point in the Discussion, we have focussed on metal uptake by roots. Foliar metal uptake may also occur, directly from the water column; plant–sediment relationships will tend to be obscured in such cases, particularly under running water conditions. The absence of a relationship between metal concentrations in plants and those in sediment interstitial water for certain plants, for particular metals, or, as is the case here, for some sampling stations may well reflect an important foliar contribution to metal bioaccumulation. Once general relationships have been established between plant metal levels and sediment metal

Fig. 5. Relationships between Cu concentrations (±SD) in leaves of *V. americana* collected in 1990 (○, Lake St. Louis; ●, Lake St. Pierre) versus (*a*) [Cu(S3)] in the sediments, (*b*) total Cu concentrations in the sediments of Lake St. Louis only, and (*c*) total Cu concentrations in the sediments of Lake St. Pierre only. In Fig. 5*c*, station 49 has been removed from the statistical calculation because of possibly elevated foliar uptake directly from the water column (see text).



concentrations, outlier stations showing higher than predicted (foliar) metal levels could be taken as an indication of the possible presence of local point sources of (dissolved) metal contamination.

As an example of this approach, consider the relationship between Cu concentrations in leaves of *Vallisneria* collected in Lake St. Pierre and Lake St. Louis in 1990 and the extractable sediment Cu concentration, [Cu(S3)] (Fig. 5*a*). In Lake St. Louis, there was a general enrichment of Cu in the leaves of the plants compared with those from Lake St. Pierre, particularly for the plants growing in the waters derived from the Ottawa River that arrive via the Lake des Deux Montagnes (stations L1, L4, and L9). In Lake St. Pierre, *Vallisneria* leaf [Cu] from station 49 is much higher than the other concentrations observed for plants in this lake. At stations L1, L4, L9, and 49, the extractable Cu concentrations in the sediments were low, whereas the Cu concentrations in the leaves were the highest, suggesting a Cu contribution from the surrounding water. We suspect that this foliar accumulation, combined with probable basipetal translocation of Cu, contributed to the high variability in the root Cu data and attenuated potential relationships between [Cu] in roots and [Cu] in the sediments.

Some of our sampling stations lie within the plumes of contaminated effluents; the contaminated water column at these sites would be expected to contribute to enhanced foliar uptake of metals. For example, at station 49 of Lake St. Pierre, visited during the 1990 sampling campaign, many metal concentrations in the leaves of *Vallisneria* did not fit the general distribution when related to metal concentrations in the roots or in the sediments. This sampling station, where plants were rooted on a very rough, irregular substrate, is located near the outlet of the Richelieu River and received the contaminated effluent discharges of the Sorel-Tracy industrial complex, which contributed high loads of Cr, Cu, Ni, Pb, and Zn to the lake (Sylvestre et al. 1992). We suspect that concentrations of these metals in the water column were higher there than at the other stations sampled in the same lake and that metal contributions from the surrounding water via leaf uptake were correspondingly higher at this station.

In Lake St. Pierre, stations with the highest sediment metal concentrations do not correspond to those showing the maximum plant metal levels. This lack of correspondence reflects the prevailing hydrodynamics. At station 49, where high metal concentrations are expected in the water column due to the proximity of the effluent discharges, the sediments did not have a high metal content. These sediments were very hard and had a low binding capacity (88% sand, 6% loam, 6% clay, <1% organic matter), as anticipated for a region with high stream velocities. The effluent discharges flow through the southern portion of Lake St. Pierre (Boudreau et al. 1994), and with the reduction in stream velocity, the metals in the water can be deposited and incorporated into the sediments, as, for example, at station 26, located in the southern part of Lake St. Pierre (Fig. 1), where the sediments offer a higher binding capacity (27% sand, 46% loam, 27% clay, 5% organic matter). In lakes, under lentic conditions, one normally expects to find a relationship between [M] in the water column and [M] in sediments because of the establishment of an equilibrium between metals in the dissolved and adsorbed phases. However, for running water conditions such as those encountered in the St. Lawrence River, high point-source metal concentrations in the water do not necessarily give rise to metal contamination of the bottom sediments.

Vallisneria as a biomonitor

Biomonitor species are commonly used to determine if contaminants are bioavailable and thus potentially toxic to the indigenous biota (St-Cyr et al. 1997). The results of the present study suggest that *Vallisneria* could be used in the St. Lawrence River as a biomonitor species to monitor metal contamination in the sediments, particularly for Cd, Pb, and Zn. This plant is the dominant submerged species in the St. Lawrence River. It occurs widely and grows in most submerged plant communities and in all water types encountered in the St. Lawrence River. Also, *Vallisneria* concentrates metals (other than Pb) to higher levels in its above-ground parts (leaves) than do the other major submerged rooted species present in the St. Lawrence River plant communities (St-Cyr and Campbell 1994).

In the context of a biomonitoring scheme designed to integrate the overall impact of metal contamination, metal uptake by both plant roots and plant foliage represents an advantage. Roots are better bioindicator organs than shoots for detecting metal contamination in sediments, particularly in running waters. However, before use, roots must be carefully cleaned with proven safe methods to remove the important iron oxyhydroxide plaque deposited on their surfaces, without attacking the underlying root tissue. This latter recommendation can probably be extended to most other aquatic plant species.

Acknowledgements

This work was supported by a joint grant to P.G.C. Campbell from the Natural Sciences and Engineering Research Council of Canada and the St. Lawrence Centre, Environment Canada. Financial support to L. St-Cyr (INRS postdoctoral fellowship) is gratefully acknowledged. We thank SCUBA divers R. Rodrique, F. Fournier, P. Goupil, and A. Vachon and laboratory technicians M. Geoffroy-Bordeleau and L. Fraser for their contributions. L. St-Cyr would like to address a very special thank you to Dr. Pierre Jolicoeur of the University of Montreal, recently deceased, for his invaluable insight into the use of statistics in biology.

References

Adams, D.D. 1994. Sediment pore water sampling. *In* Handbook of techniques for aquatic sediments sampling. 2nd ed. *Edited by* A. Mudroch and S.D. MacKnight. Lewis Publishers, Boca Raton, Fla. pp. 171–202.

Boudreau, P., Leclerc, M., and Fortin, G.R. 1994. Modélisation hydrodynamique du lac Saint- Pierre, fleuve Saint-Laurent: l'influence de la végétation aquatique. Can. J. Civ. Eng. **21**: 471–489.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. *In* Metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D.R. Turner. John Wiley & Sons Ltd., Chichester, U.K. pp. 45–102.

Campbell, P.G.C., and Lewis, A.G. (*Editors*). 1988. Biologically available metals in sediments. Assoc. Comm. Sci. Criteria Environ. Qual. Publ. No. 27694. National Research Council of Canada, Ottawa, Ont.

Campbell, P.G.C., and Tessier, A. 1996. Ecotoxicology of metals in the aquatic environment: geochemical aspects. *In* Ecotoxicology. A hierarchical treatment. *Edited by* M.C. Newman and C.H. Jagoe. Lewis Publishers, Boca Raton, Fla. pp 11–58.

Campbell, P.G.C., Tessier, A., Bisson, M., and Bougie, M. 1985. Accumulation of copper and zinc in the yellow water lily, *Nuphar variegatum*: relationships to metal partitioning in the adjacent lake sediments. Can. J. Fish. Aquat. Sci. **42**: 23–32.

Good, B.J., and Patrick, W.H., Jr. 1987. Root–water–sediment interface processes. *In* Aquatic plants for water treatment and resource recovery. *Edited by* K.R. Reddy and W.H. Smith. Magnolia Publishing Inc., Bellevue, Wash. pp. 359–371.

Guilizzoni, P. 1991. The role of heavy metals and toxic materials in the physiological ecology of submerged macrophytes. Aquat. Bot. **41**: 87–109.

Jackson, L.J. 1998. Paradigms of metal accumulation in rooted aquatic vascular plants. Sci. Total Environ. **219**: 223–231.

Jackson, L.J., Rasmussen, J.B., Peters, R.H., and Kalff, J. 1991. Empirical relationships between the element composition of aquatic macrophytes and their underlying sediments. Biogeochemistry, **12**: 71–86.

Jackson, L.J., Kalff, J., and Rasmussen, J.B. 1993. Sediment pH and redox potential affect the bioavailability of Al, Cu, Fe, Mn, and Zn to rooted aquatic macrophytes. Can. J. Fish. Aquat. Sci. **50**: 143–148.

Jolicoeur, P. 1997. Introduction à la biométrie. 3^{e} éd. Décarie Éditeur inc., Mont-Royal, Qué.

Luoma, S.N. 1989. Can we determine the biological availability of sediment-bound trace elements? Hydrobiologia, **176/177**: 379–396.

Otte, M.L., Rozema, J., Koster, L., Haarsma, M.S., and Broekman, R.A. 1989. Iron plaque on roots of *Aster tripolium* L.: interaction with Zn uptake. New Phytol. **111**: 309–317.

Pedersen, O., and Sand-Jensen, K. 1993. Water transport in submerged macrophytes. Aquat. Bot. **44**: 385–406.

Peech, M. 1965. Hydrogen-ion activity. *In* Methods of soil analysis. Part 2. Chemical and microbiological properties. *Edited by* C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger, and F.E. Clark. American Society Agronomy, Inc., Madison, Wis. pp. 914–926.

Rattray, M.R., Howard-Williams, C., and Brown, J.M.A. 1991. Sediment and water as sources of nitrogen and phosphorus for submerged rooted aquatic macrophytes. Aquat. Bot. **40**: 225–237.

St-Cyr, L., and Campbell, P.G.C. 1994. Trace metals in submerged plants of the St. Lawrence River. Can. J. Bot. **72**: 429–439.

St-Cyr, L., and Campbell, P.G.C. 1996. Metals (Fe, Mn, Zn) in the root plaque of submerged aquatic plants collected in situ: relations with metal concentrations in the adjacent sediments and in the root tissue. Biogeochemistry, **33**: 45–76.

St-Cyr, L., Campbell, P.G.C., and Guertin, K. 1994. Evaluation of the role of submerged plant beds in the metal budget of a fluvial lake. Hydrobiologia, **291**: 141–156.

St-Cyr, L., Cattaneo, A., Chassé, R., and C.G.J. Fraikin. 1997. Technical evaluation of monitoring methods using macrophytes, phytoplankton and periphyton to assess the impacts of mine effluents on the aquatic environment. Technical report for the Aquatic Effects Technology Evaluation (AETE) Program, AETE Project 2.3.2, prepared for Natural Resources Canada, Canada Centre for Mineral and Energy Technology (CANMET), Ottawa, Ont.

Sylvestre, A., Champoux, L., and Leclair, D. 1992. Synthèse des connaissances sur les aspects physiques et chimiques de l'eau et des sédiments du lac Saint-Pierre. Rapp. tech. ZIP n° 11. Environnement Canada, Centre Saint-Laurent, Montréal, Qué.

Taylor, G.J., and Crowder, A.A. 1983. Use of the DCB technique for extraction of hydrous iron oxides from roots of wetland plants. Am. J. Bot. **70**: 1254–1257.

Tessier, A. 1992. Sorption of trace elements on natural particles in oxic environments. *In* Environmental particles. Vol. 1. Environmental, analytical and physical chemistry series. *Edited by* J. Buffle and H.P. Van Leeuwen. Lewis Publishers, Boca Raton, Fla. pp. 425–453.

Tessier, A., Campbell, P.G.C., and Bisson, M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. **51**: 844–851.

Tessier, A., Couillard, Y., Campbell, P.G.C., and Auclair, J.C. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnol. Oceanogr. **38**: 1–17.

Welsh, R.P.H., and Denny, P. 1980. The uptake of lead and copper by submerged aquatic macrophytes in two English lakes. J. Ecol. **68**: 443–455.

Cumulative effects of pulp mill and municipal effluents on epilithic biomass and nutrient limitation in a large northern river ecosystem

Garry J. Scrimgeour and Patricia A. Chambers

Abstract: Large-scale patterns in epilithic biomass and nutrient status were evaluated at 33 sites located upstream and downstream of point-source anthropogenic effluents in the Athabasca and Wapiti–Smoky rivers in Alberta, Canada. Multiple regression showed that epilithic chlorophyll *a* was significantly ($p < 0.0001$) related to concentrations of dissolved inorganic nitrogen and marginally ($p = 0.06$) significantly related to soluble reactive phosphorus. Epilithic biomass was up to 50 times higher immediately downstream of point-source inputs compared with sites upstream and those 20–150 km downstream. Data from nutrient diffusing substrata showed that the epilithon at 18 of the 33 sites was nutrient limited, while 14 sites showed no nutrient limitation; interpretation of the remaining site was inconclusive. Of the 18 nutrient-limited sites, six were nitrogen limited, five were phosphorus limited, and seven were co-limited. Multiple discriminant function analysis showed that the combined concentration of soluble reactive phosphorus and dissolved inorganic nitrogen was a significant discriminator between deplete and replete sites.

Résumé : Nous avons évalué les patrons à grande échelle de la biomasse épilithique et de la situation des nutriants à 33 sites se trouvant en amont et en aval d'effluents de sources ponctuelles anthropiques dans les rivières Athabasca et Wapiti–Smoky, en Alberta (Canada). Une régression multiple a montré que la chlorophylle *a* épilithique était liée de façon significative ($p < 0{,}0001$) aux concentrations d'azote inorganique dissous et de façon marginale ($p = 0{,}06$) au phosphore réactif soluble. La biomasse épilithique était jusqu'à 50 plus élevée juste en amont des intrants ponctuels par rapport aux sites d'amont et à ceux situés de 20 à 150 km en aval. Les données obtenues sur les substrats diffusant des nutriants ont montré que, à 18 des 33 sites, l'épilithon était limité en nutriants, tandis qu'aucune limitation des nutriants n'apparaissait à 14 des sites; l'interprétation n'était pas concluante pour le dernier site. Sur les 18 sites limités en nutriants, six étaient limités en azote, cinq en phosphore et sept en ces deux éléments. L'analyse discriminante a montré que les concentrations combinées de phosphore réactif soluble et d'azote inorganique dissous constituaient un discriminateur significatif entre les sites appauvris et riches en nutriants.

[Traduit par la Rédaction]

Introduction

Eutrophication caused by excessive inputs of N and P is the most common impairment of surface waters throughout the world. For instance, in the United States, 50% of the lake area and 60% of the river reaches are deemed to be unsuitable for their designated use (e.g., drinking, irrigation, industry, recreation, or fishing) due to eutrophication (U.S. Environmental Protection Agency 1990). The continuing concern about widespread deterioration in water quality due to nutrients has resulted in considerable effort directed at research on N and P cycling and food web responses in lakes, the development of models relating nutrients to primary production, and the formation of management strategies to control nutrient loading to lakes (e.g., Carpenter and Cottingham 1997). By comparison, the effects of nutrient pollution on streams and rivers, large rivers in particular, have been little investigated and our predictive capability is limited.

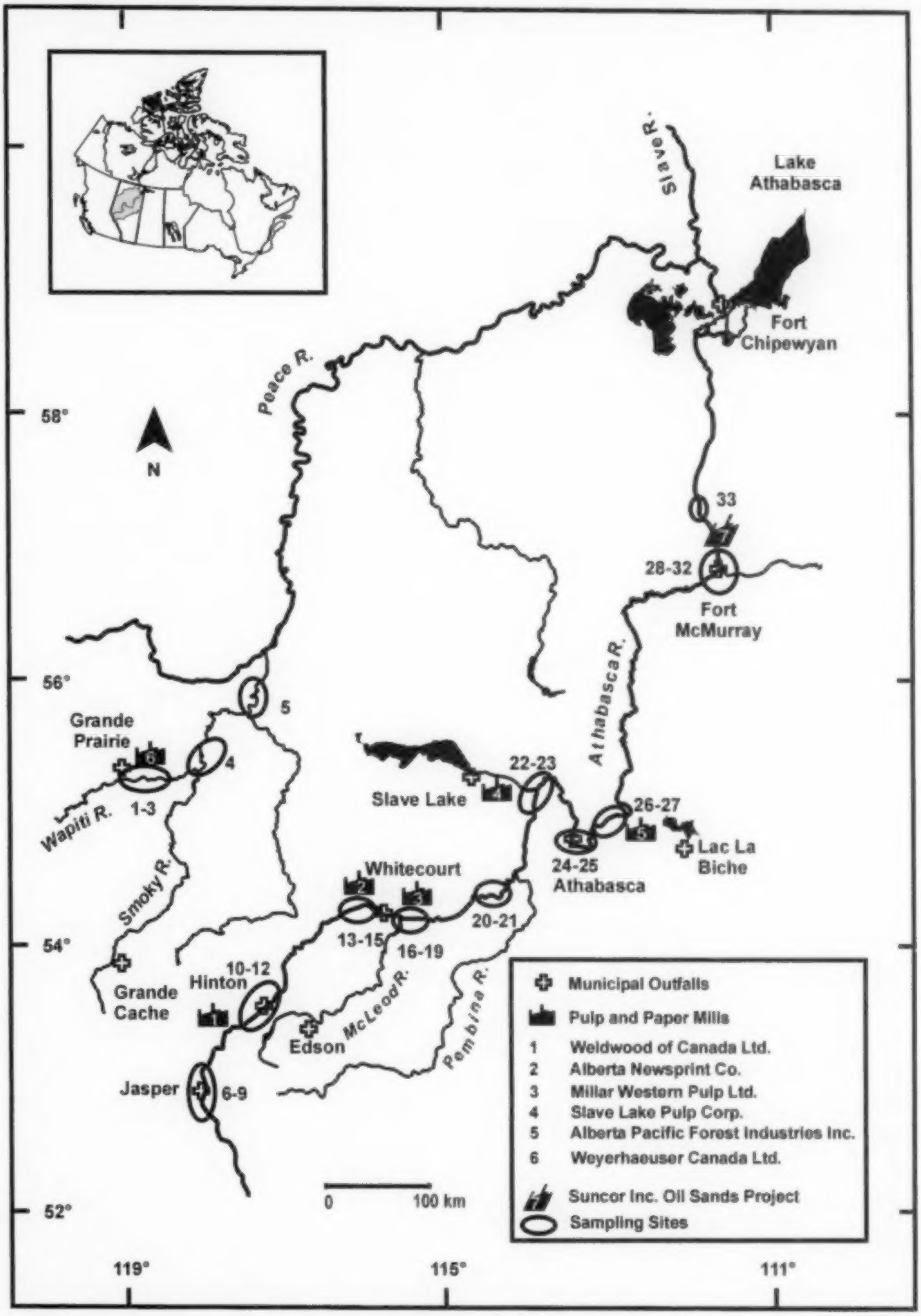
Investigations into the effects of nutrient addition on lotic ecosystems have largely focused on nutrient dynamics (e.g., spiralling, exchanges amongst nutrient pools) or the mechanisms regulating nutrient-mediated responses of aquatic biota. These studies, completed almost exclusively in small streams with little or no anthropogenic disturbances, indicate that P supply is largely controlled through geomorphic processes (weathering, erosion) whereas N is derived from biological processes (instream and the drainage basin) and atmospheric exchange. Using a diversity of approaches, these studies have shown that periphyton growth is often limited by insufficient N or P (and occasionally micronutrients) and that nutrient limitation may be temporally variable and masked by light limitation, the frequency of floods, or grazing (e.g., Lohman et al. 1992; Mulholland et al. 1995; Wold and Hershey 1999). Despite the many controlled experiments on the effects of nutrient manipulation on periphyton abundance, few studies have evaluated the effects of

Received July 5, 1999. Accepted February 2, 2000.
J15237

G.J. Scrimgeour.[1] Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.
P.A. Chambers.[2] National Water Research Institute, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada.

[1] Author to whom all correspondence should be sent at the following address: Forest Resources Business Group, Alberta Research Council, P.O. Bag 4000, Vegreville, AB T9C 1T4, Canada. e-mail: gscrimgeour@arc.ab.ca

Fig. 1. Location of municipalities, pulp mills, and other industries with continuous or consecutive 2-weekly wastewater discharges and study sites in the Wapiti–Smoky and Athabasca river basins.


Slave R.
Lake Athabasca
Fort Chipewyan
Peace R.
58°
N
33
28-32
Fort McMurray
Athabasca R.
56°
5
Grande Prairie
4
1-3
Wapiti R.
Smoky R.
Slave Lake
22-23
26-27
24-25
Athabasca
Lac La Biche
Whitecourt
13-15
16-19
20-21
54°
10-12
Hinton
Grande Cache
Edson
McLeod R.
Pembina R.
Jasper
6-9
0
100 km
52°
119°
115°
111°
Municipal Outfalls
Pulp and Paper Mills
1 Weldwood of Canada Ltd.
2 Alberta Newsprint Co.
3 Millar Western Pulp Ltd.
4 Slave Lake Pulp Corp.
5 Alberta Pacific Forest Industries Inc.
6 Weyerhaeuser Canada Ltd.
Suncor Inc. Oil Sands Project
Sampling Sites

point-source inputs on periphyton abundance and nutrient status over large longitudinal gradients.

In the present study, we examined spatial patterns in nutrient loading, epilithic biomass, and epilithic nutrient limitation in two large, mountain-fed river systems in northwestern Canada. The Wapiti–Smoky and Athabasca rivers, Alberta, re-

Table 1. Mean effluent discharge and TN and TP loads for municipalities, pulp mills, and other industries with continuous or biweekly discharges to the Wapiti–Smoky and Athabasca rivers and their tributaries.

Location	Effluent source	Receiving water	Discharge (m^3·day^{-1})	TN (kg·day^{-1})	TP (kg·day^{-1})
Wapiti–Smoky rivers					
Grande Prairie	Grande Prairie STP	Wapiti River	10 728	249	53
	Weyerhaeuser Canada BKPM	Wapiti River	58 633	469	72
Grande Cache	Grande Cache STP	Smoky River	2032	32	8
	Alberta Power Ltd. H.R. Milner Station	Smoky River	1469	1[a]	0.2
Athabasca River					
Jasper	Jasper STP	Athabasca River	3948	79	17
Hinton	Weldwood of Canada Ltd. BKPM	Athabasca River	106 557[b]	535	79
Edson	Edson STP	McLeod River	3954	61	16
Whitecourt	Alberta Newsprint Co. CTMPM	Athabasca River	15 536	94	92
	Millar Western Pulp Ltd. CTMPM	Athabasca River	12 041	124	34
	Whitecourt STP	Athabasca River	3417	61	12
Slave Lake	Slave Lake STP	Lesser Slave River	2729	62	9
	Slave Lake Pulp Corp. CTMPM	Lesser Slave River	4698	103	54
Athabasca	Athabasca STP	Athabasca River	952	25	5
	Alberta Pacific Forest Industries BKPM	Athabasca River	67 826	177	72
Lac La Biche	Lac La Biche	Field Lake	1425	33	6
Fort McMurray	Fort McMurray STP	Athabasca River	14 000	344	27
	Suncor Inc. Athabasca Oil Sands Project	Athabasca River	35 020	42	7
Fort Chipewyan	Fort Chipewyan STP	Riviere des Rochers	na	na	na

Note: STP, sewage treatment plant; BKPM, bleached kraft pulp mill; CTMPM, chemithermomechanical pulp mill; na, data not available.

[a] Total Kjeldahl N.

[b] Hinton municipal sewage is combined and discharged with Weldwood effluent. All data are summarized in Chambers (1996). All discharges were operating during the early fall – late fall, 1994. Discharge from the Grande Prairie STP to the Wapiti River is discontinuous where a 2-week period of discharge is followed by a 2-week period of no discharge.

ceive a variety of effluents including municipal sewage and pulp mill waste water. Our objectives were to (*i*) identify spatial variation in epilithic biomass in relation to point-source and tributary inputs, (*ii*) develop empirical models relating epilithic biomass to water quality (nutrients, irradiance, temperature), and (*iii*) assess nutrient limitation of the epilithon and its predictability based on water chemistry. Our approach combined observations of epilithic biomass and water chemistry with in situ nutrient limitation experiments conducted at 33 sites located upstream and downstream of anthropogenic point-source and tributary inputs. This approach of in situ observation and experimentation allowed us to assess the cumulative impacts of multiple pollutant sources on the magnitude and downstream extent of nutrient enrichment in these two large rivers.

Materials and methods

Study area

The Athabasca River (drainage area 155 000 km^2) originates in the Rocky Mountains of west-central Alberta and then flows northeast across the boreal plains ecoregion (Strong and Leggat 1992) where luvisolic soils predominate (Fig. 1). Mean daily flows at the Town of Athabasca average 407 m^3·s^{-1} (1980–1993), with peak flows typically occurring in June after snowmelt (June monthly mean, 1980–1993, 1016 m^3·s^{-1}) and lowest flows in February (February monthly mean, 1980–1993, 62 m^3·s^{-1}) (Environment Canada 1994). The Wapiti River arises in the Rocky Mountains of east-central British Columbia and then flows east across the boreal plains of Alberta and converges with the Smoky River 42 km downstream of the City of Grande Prairie (Fig. 1). After convergence with the Wapiti River, the Smoky River drains into the Peace River. Mean daily flows for the Wapiti River at Grande Prairie average 88 m^3·s^{-1} (1980–1993), with peak flows occurring in June after snowmelt (June monthly mean, 1980–1993, 297 m^3·s^{-1}) and lowest flows in February (February monthly mean, 1980–1993, 12 m^3·s^{-1}) (Environment Canada 1994).

Two bleached kraft pulp mills (BKPM) and three chemithermomechanical pulp mills (CTMPM) currently operate in the Athabasca River basin; one BKPM operates in the Wapiti River basin (Fig. 1; Table 1). There are also two oil sands projects in the Athabasca basin (but only one with continuous discharge of utility waste water) and a power station that releases utility waste water to the Smoky River. Sewage is released continuously to the Athabasca River and its tributaries from eight communities (as well as the Town of Hinton, which discharges its sewage with the Hinton BKPM) and to the Wapiti–Smoky rivers from two communities. All eight inputs released effluents to the Wapiti and Athabasca rivers between early and late fall of 1994. Other activities include small communities with sewage lagoons that release once or twice a year (i.e., spring and (or) fall) and coal mines, gas plants, and gravel-washing operations that have little or no discharge.

Water chemistry

Samples for water chemistry were collected twice in the fall of 1994 when nutrient diffusing substrata (NDS) were deployed and retrieved (Table 2). Instantaneous estimates of pH and water temperature were obtained with a Fisher Scientific Accumet 1000 series handheld meter. Water samples for dissolved oxygen were collected in 500-mL dissolved oxygen bottles and analysed according to Carpenter's (1965) modified Winkler technique. Samples for soluble reactive phosphorus (SRP) (detection limit 0.5 μg·L^{-1}) were collected in 500-mL Nalgene polyethylene bottles, stored on

Table 2. Location of study sites in the Wapiti–Smoky and Athabasca river basins. River distances in the Wapiti–Smoky and Athabasca rivers are expressed as distances downstream of the uppermost sites (i.e., site 1 on the Wapiti River and site 6 on the Athabasca River).

Site	Location	River distance (km)
Wapiti–Smoky rivers		
1	3 km upstream Grande Prairie STP	0
2	4.5 km downstream Grande Prairie STP	8
3	11.5 km downstream Grande Prairie STP; 2 km downstream Weyerhaeuser Canada BKPM	15
4	40 km downstream Weyerhaeuser Canada BKPM; 12 km downstream Wapiti–Smoky rivers confluence	43
5	135 km downstream Weyerhaeuser Canada BKPM; 94 km downstream Wapiti–Smoky rivers confluence	138
Athabasca River		
6	0.7 km upstream Japer STP	0
7	2.5 km downstream Jasper STP	3
8	4 km downstream Jasper STP	5
9	20 km downstream Jasper STP	21
10	1 km upstream Weldwood of Canada Ltd. BKPM	82
11	1 km downstream Weldwood of Canada Ltd. BKPM	84
12	20 km downstream Weldwood of Canada Ltd. BKPM	103
13	120 km downstream Weldwood of Canada Ltd. BKPM	219
14	150 km downstream Weldwood of Canada Ltd. BKPM; 30 km upstream Alberta Newsprint Co. CTMPM at Whitecourt	255
15	1.5 km downstream Alberta Newsprint Co. CTMPM; 8.5 km upstream Millar Western Forest Products CTMPM; 12.5 km upstream Whitecourt STP	287
16	McLeod River 2.5 km upstream confluence with Athabasca River	—
17	12 km downstream Alberta Newsprint Co. CTMPM; 2 km downstream Millar Western Forest Products CTMPM; 2 km upstream Whitecourt STP	294
18	9 km downstream Whitecourt STP	305
19	20 km downstream Whitecourt STP	316
20	70 km downstream Whitecourt STP	388
21	94 km downstream Whitecourt STP	412
22	10 km upstream of confluence with Lesser Slave River	520
23	3 km downstream from confluence with Lesser Slave River	533
24	1 km upstream Athabasca STP	637
25	1 km downstream Athabasca STP	639
26	3 km downstream Athabasca STP; 12 km upstream Alberta Pacific Forest Industries BKPM	641
27	4 km downstream Alberta Pacific Forest Industries BKPM	657
28	4 km upstream Fort McMurray STP	1028
29	Clearwater River 7 km upstream confluence with Athabasca River	—
30	1 km upstream Fort McMurray STP; 1 km downstream Clearwater River confluence	1031
31	1 km downstream Fort McMurray STP	1033
32	3 km downstream Fort McMurray STP	1035
33	60 km downstream Fort McMurray STP	1092

Note: STP, sewage treatment plant; BKPM, bleached kraft pulp mill; CTMPM, chemithermomechanical pulp mill.

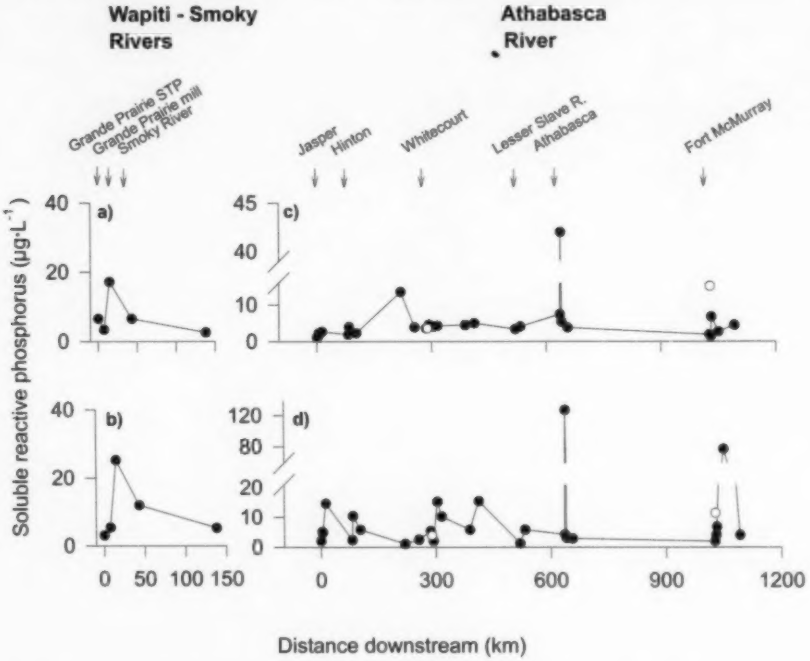
ice in the field, and then refrigerated at 4°C in the laboratory until analysed following the molybdenum blue technique (American Public Health Association 1975). Samples for nitrite plus nitrate ($NO_2 + NO_3$) and ammonium (NH_4^+) (detection limits 1 $\mu g \cdot L^{-1}$) were collected in 50-mL polystyrene bottles, stored on ice in the field, and then refrigerated at 4°C in the laboratory. $NO_2 + NO_3$ samples were filtered through prewashed 0.45-µm HAWP Millipore membrane filters. $NO_2 + NO_3$ and NH_4^+ concentrations were analysed on a Technicon autoanalyzer (Stainton et al. 1977) and combined to produce dissolved inorganic nitrogen (DIN). Samples for SRP and DIN were analysed within 24 h of collection.

Light attenuation coefficients were calculated for all 33 study sites upon placement and retrieval of NDS to determine whether variation in epilithic biomasses in the Wapiti–Smoky and Athabasca rivers relates to light availability. For each site, incident light at the water surface (microsiemens per square centimetre) was compared with that at 10-cm intervals over a 60-cm vertical transect through the water column with a Licor quantum sensor. Light attenuation coefficients were calculated as the natural log of surface light versus depth using linear regression.

Epilithic biomass

Samples for epilithic biomass determination were collected from natural substrata at each site upon placement and retrieval of NDS (approximate widths, lengths, and heights = 10 × 15 × 7 cm, respectively). Individual stones were collected randomly from one large riffle area (75 m long) at each site where water depths ranged from 0.25 to 0.65 m, and current velocities were 0.15–0.65 $m \cdot s^{-1}$. A single 9.6-cm^{-2} area was scraped from the upper surface of each of the 10 stones from each site and immediately frozen at about −10°C with dry ice. Epilithic biomass, expressed as chlorophyll *a* (Chl *a*), for individual stone scrapings was determined following

Fig. 2. Concentrations of SRP at 33 sites in early fall (September–October) (top panels) and late fall (October–November) (bottom panels) in the (*a* and *b*) Wapiti–Smoky and (*c* and *d*) Athabasca rivers, 1994. Solid circles are sites on the mainstem of the rivers; open circles are sites on the McLeod and Clearwater river tributaries of the Athabasca drainage basin. Note the high concentrations at site 25 in early fall (42 $\mu g \cdot L^{-1}$) and late fall (127 $\mu g \cdot L^{-1}$) and at site 31 in late fall (77 $\mu g \cdot L^{-1}$). Arrows indicate main anthropogenic inputs. Downstream distances represent distances downstream of the uppermost sites in both the Wapiti–Smoky and Athabasca rivers. Note the differences in the *x*-axis and *y*-axis scales between the Wapiti–Smoky and Athabasca rivers.



the method of Nusch (1980) and the concentration determined on a Turner Designs model 10 series fluorometer. Water samples for determination of sestonic Chl *a* were collected in 500-mL Nalgene bottles and determined following the ethanol extraction technique reported by Bierhuizen and Prepas (1985).

Diffusing substrata

Spatial patterns in nutrient limitation in the Wapiti–Smoky and Athabasca rivers were investigated using NDS previously designed for use in these rivers (Scrimgeour and Chambers 1997). Each NDS consisted of a porous clay pot (height = 6 cm, width = 11 cm, volume = 325 mL) filled with agar and the test compound and sealed with a 4-mm polypropylene base (diameter = 12 cm) using aquarium safe silicone sealant. NDS were prepared as described by Scrimgeour and Chambers (1997) and filled with a hot solution containing agar (20 $g \cdot L^{-1}$ in autoclaved deionized–distilled water) mixed with one of the test compounds: N (0.8 M $NaNO_3$), P (0.5 M KH_2PO_4), or N (0.8 M $NaNO_3$) + P (0.5 M KH_2PO_4) enriched or unamended controls.

NDS were deployed between August 26 and September 16, 1994, and removed 14–31 days later (mean ± 1 SE = 20 ± 1 days), which is sufficiently long for algal colonisation and accrual (Scrimgeour and Chambers 1997; Francoeur et al. 1999). During removal, epilithic biomass was scraped from a 9.6-cm^2 area from each NDS, frozen immediately, and later analysed for Chl *a* as described for stone scrapings. While 10 replicates of each NDS treatment were deployed, retrieval of between eight and 10 replicates of each treatment resulted in variable numbers of treatment replicates.

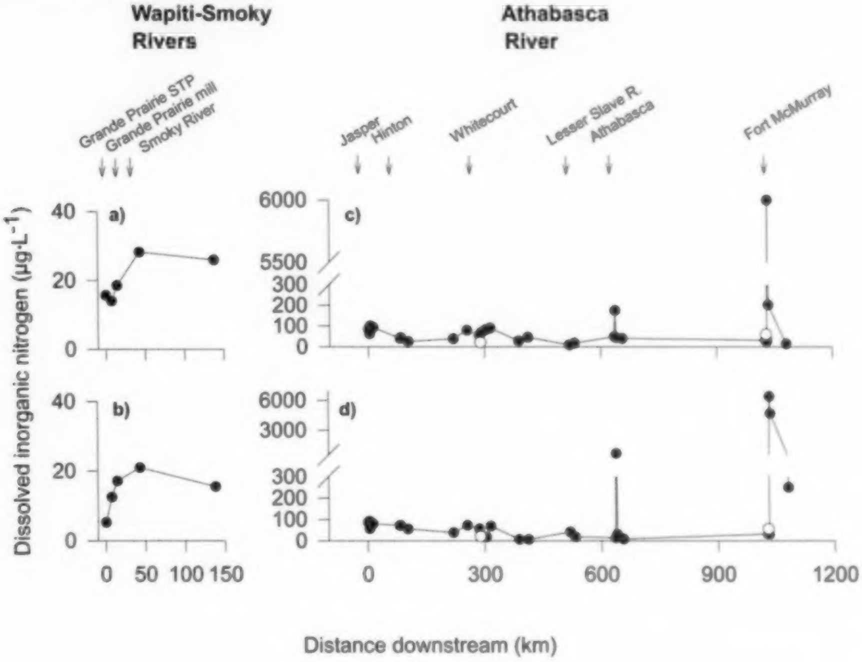
We compared patterns in nutrient limitation observed using NDS with those expected from water column concentrations of DIN and SRP and DIN:SRP ratios and assumed that concentrations of 55 μg $DIN \cdot L^{-1}$ and 5 μg $SRP \cdot L^{-1}$ are sufficient to satisfy algal requirements of moderately dense mats (Grimm and Fisher 1986; Perrin et al. 1987; Bothwell 1989, 1992). We also assumed that low (i.e., <10), intermediate (i.e., 10–20) and high (i.e., >20) DIN:SRP molar ratios suggest that the algal community is N limited, co-limited, and P limited, respectively (Schantz and Juon 1983; Lohman et al. 1991; Wold and Hershey 1999).

Experimental design and statistical analyses

Differences among sites in epilithic Chl *a* from stone scrapings were tested with a single-factor ANOVA after $\log_{10}$ transformation to remedy inequality of variances and performed separately for samples collected in early and late fall. We estimated the distance that anthropogenic inputs alter algal biomass by comparing biomass upstream of the anthropogenic input with that downstream. Where differences existed in algal biomass between the upstream and downstream sites, we defined the distance from the point-source input to the downstream site as the minimum distance of point-source effects on algal biomass. Similarly, we estimated the minimum distance of point-source effects on N and P deficiency in the Athabasca River by calculating the distance between the point-source input and the downstream site that differed in its nutrient limitation status from that immediately upstream of the point-source input. This analysis was not completed for the Wapiti–Smoky rivers because of low numbers of study sites.

Multiple regression using a backward-elimination approach (elimination level $F = 0.10$) was used to relate epilithic biomass to SRP, DIN, light attenuation coefficient, and instantaneous water

Fig. 3. Concentrations of DIN at 33 sites in early fall (September–October) (top panels) and late fall (October–November) (bottom panels) in the (*a* and *b*) Wapiti–Smoky and (*c* and *d*) Athabasca rivers, 1994. Solid circles are sites on the mainstem of the rivers; open symbols are sites on the McLeod and Clearwater river tributaries of the Athabasca drainage basin. Note the high concentrations at site 25 in late fall (640 $\mu g \cdot L^{-1}$) and at sites 31 and 32 in early fall (site 31, 6000 $\mu g \cdot L^{-1}$; site 32, 202 $\mu g \cdot L^{-1}$) and late fall (site 31, 6400 $\mu g \cdot L^{-1}$; site 32, 4686 $\mu g \cdot L^{-1}$). Arrows indicate main anthropogenic inputs. Downstream distances represent the distances downstream of the uppermost sites in both the Wapiti–Smoky and Athabasca rivers. Note the differences in the *x*-axis and *y*-axis scales between the Wapiti–Smoky and Athabasca rivers.



temperature to quantify how well these variables could predict variation in epilithic biomass in the early and late fall.

Single-factor ANOVAs were performed to test for treatment effects on NDS at the 33 sites. When single-factor ANOVA tests indicted a significant treatment effect, pairwise means comparisons were completed using least squares means tests with Bonferroni-adjusted alphas.

Differences in water chemistry and physicochemical variables between nutrient-limited and nutrient-nonlimited sites were initially compared using *t* tests to determine whether they differed between nutrient-limited and nutrient-nonlimited sites. These analyses were followed by a more conservative multivariate discrimination function analysis. For each of these variables, we calculated a mean for each site based on measurements taken when NDS were deployed and subsequently removed from the river. Prior to completing the discriminant function analysis, a correlation matrix was completed to ensure that each of the variables was statistically independent. This analysis indicated that epilithic Chl *a* was related to combined concentrations of SRP + DIN ($r = 0.75$) and was excluded from the discriminant function analyses.

A forward-selection approach was used in the discriminant function analysis. An entry and removal criterion of $F = 0.10$ was performed initially to select statistically significant variables (Tabachnick and Fidell 1983). Nonredundant variables were entered into a canonical discriminant function model analysis to determine if the physicochemical characteristics of nutrient-replete and nutrient-deplete sites differed and to quantify the predictability of group membership.

For all analyses, Shapiro–Wilks tests were used to determine whether data were normally distributed, and homogeneity of variances was tested with *F* tests (two-sample *t* tests) and Bartletts test and by graphically examining the residuals (following the ANOVA tests). Where variances were heterogeneous, data were transformed to satisfy data normality and homogeneity of variance assumptions. All statistical comparisons were conducted with SAS (SAS Institute Inc. 1987), with results presented as means ± 1 SE unless stated otherwise.

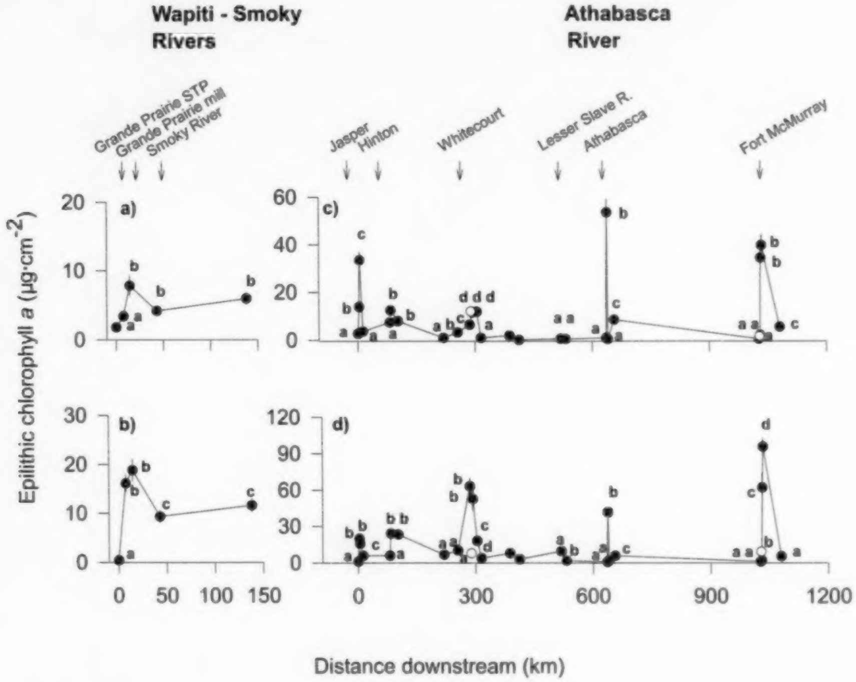
Results

Water chemistry and clarity

Instantaneous water temperatures in the Wapiti–Smoky and Athabasca rivers ranged from 5.5 to 12.5°C in early fall but declined to 0.1–2.0°C by late fall. Concentrations of dissolved oxygen typically approached or exceeded saturation with respect to temperature and elevation at all sites. pH levels were slightly alkaline, ranging from 7.4 to 8.3 and from 7.6 to 8.4 in early and late fall, respectively, and irradiance reaching the river bottom at depths of 75 cm exceeded 1% of surface irradiance.

In the Wapiti–Smoky rivers, SRP ranged from 3 to 6 $\mu g \cdot L^{-1}$ upstream of point sources but was typically three times higher downstream of the sewage treatment plant (STP) and pulp mill (i.e., 17–25 $\mu g \cdot L^{-1}$ in early and late fall) (Figs. 2*a* and 2*b*). In the Athabasca River, concentrations of SRP typi-

Fig. 4. Mean (±1 SE) concentrations of epilithic Chl *a* at 33 sites in early fall (September–October) (top panels) and late fall (October–November) (bottom panels) in the (*a* and *b*) Wapiti–Smoky and (*c* and *d*) Athabasca rivers, 1994. For some sites, error bars are contained within circles. Solid circles are sites on the mainstem of the rivers; open circles are sites on the McLeod and Clearwater river tributaries of the Athabasca drainage basin. Arrows indicate main anthropogenic inputs. Downstream distances represent the distances downstream of the uppermost sites in both the Wapiti–Smoky and Athabasca rivers. Note the differences in *x*-axis and *y*-axis scales between the Wapiti–Smoky and Athabasca rivers. Circles sharing the same letters at each of the main anthropogenic inputs are not significantly different.



cally ranged from 2 to 4 $\mu g \cdot L^{-1}$ at sites immediately upstream of point-source inputs and between 4 and 15 $\mu g \cdot L^{-1}$ downstream (Figs. 2*c* and 2*d*).

Concentrations of DIN in the Wapiti–Smoky rivers ranged from 14 to 28 and from 5 to 21 $\mu g \cdot L^{-1}$ in the early and late fall, respectively, and were about twofold higher downstream of anthropogenic inputs (Figs. 3*a* and 3*b*). Irrespective of date, concentrations of DIN in the Athabasca River ranged from 25 to 100 $\mu g \cdot L^{-1}$ and generally declined between Jasper and Lesser Slave River but were four- to 120-fold higher downstream of the Athabasca and Fort McMurray STP than at upstream reference sites (Figs. 3*c* and 3*d*).

Spatial patterns in epilithic biomass

Epilithic Chl *a* concentration varied significantly among the 33 sites in early fall (ANOVA on $\log_{10}$-transformed data, $p < 0.0001$) and late fall ($p < 0.0001$) and was strongly related to the presence of STPs and pulp mills (Fig. 4).

In the Wapiti–Smoky rivers, there was no detectable effect of the STP discharge on epilithic biomass in early fall (Bonferroni adjusted, least squares means tests between sites 1 and 2, $p > 0.01$) (Fig. 4*a*) whereas in late fall, epilithic biomass downstream of the STP was significantly (Bonferroni adjusted least squares means tests, $p < 0.01$) higher and almost 20 times that immediately upstream (Fig. 4*b*). Epilithic biomass remained significantly ($p < 0.01$) higher downstream of the STP and BKPM discharges until they converged with the Smoky River (Figs. 4*a* and 4*b*).

In the Athabasca River, epilithic Chl *a* levels were generally 2–50 times higher downstream of point-source inputs (Figs. 4*c* and 4*d*). Comparisons of epilithic Chl *a* between sites upstream and downstream of point-source inputs showed that inputs, on average, significantly (Bonferroni adjusted least squares means tests, $p < 0.01$) increased biomass for about 21 km (overall range 0–60 km) (Table 3).

Epilithic biomass was significantly ($p < 0.0001$) related to DIN and marginally significantly ($p = 0.06$) related to SRP (multiple regression analysis using combined data from early and late fall: epilithic Chl $a = -25.6 + 10.1 \log_{10} \mathrm{SRP} + 17.7 \log_{10} \mathrm{DIN}$, $r^2 = 0.67$), where epilithic biomass is expressed as Chl *a* (micrograms per square centimetre) and SRP and DIN concentrations are expressed as micrograms per litre. Removal of SRP from the regressions lowered the r^2 value from 0.67 to 0.63, indicating that SRP explained a small amount of the overall variation in epilithic biomasses compared with DIN. The inability of these models to identify water temperature as a significant predictor of epilithic biomass likely arises because of the lack of appreciable variation (overall variation in mean water temperature <4°C) in water temperature among sites.

Table 3. Minimum distances that anthropogenic inputs affected algal biomass and N and P deficiency in the Wapiti–Smoky and Athabasca rivers, fall 1994. Comparisons of algal biomass were restricted to sites where significant differences existed between upstream and downstream sites.

Algal biomass

Location	Season	Affected reach	Affected distance (km)
Wapiti-Smoky rivers			
Grande Prairie	Early fall	BKPM to site 4	40
	Late fall	STP to site 4	50
Athabasca River			
Jasper	Early fall	STP to site 8	4
	Late fall	STP to site 9	20
Hinton	Early fall	BKPM to site 12	20
	Late fall	BKPM to site 12	20
Whitecourt	Early fall	CTMPM to site 18	23
	Late fall	CTMPM to site 18	23
Lesser Slave River	Early fall	—	0
	Late fall	Confluence to site 23	13
Athabasca	Early fall	STP to site 25	1
	Late fall	STP to site 25	1
Athabasca BKPM	Early fall	BKPM to site 27	4
	Late fall	BKPM to site 27	4
Fort McMurray	Early fall	STP to site 32	60
	Late fall	STP to site 32	60

N and P deficiency

Location	Site and nutrient limitation status						Affected reach	Affected distance (km)
Jasper								
Site	6	7	8	9			STP to site 8	4
Status	P limited	Nonlimited	Nonlimited	P limited				
Hinton								
Site	10	11	12	13			BPKM to site 13	120
Status	P limited	Nonlimited	Nonlimited	Nonlimited				
Whitecourt								
Site	15	17	18	19	20	21	STP to site 21	94
Status	N limited	Nlimited	Nlimited	N+P limited	N+P limited	P limited		
Lesser Slave								
Site	22	23	24	25	26		Lesser Slave confluence to site 26	
Status	N limited	Nonlimited	Nonlimited	Nonlimited	Nonlimited			111
Fort McMurray								
Site	28	30	31	32			STP to site 33	60
Status	N+P limited	Nonlimited	Nonlimited	Nonlimited				

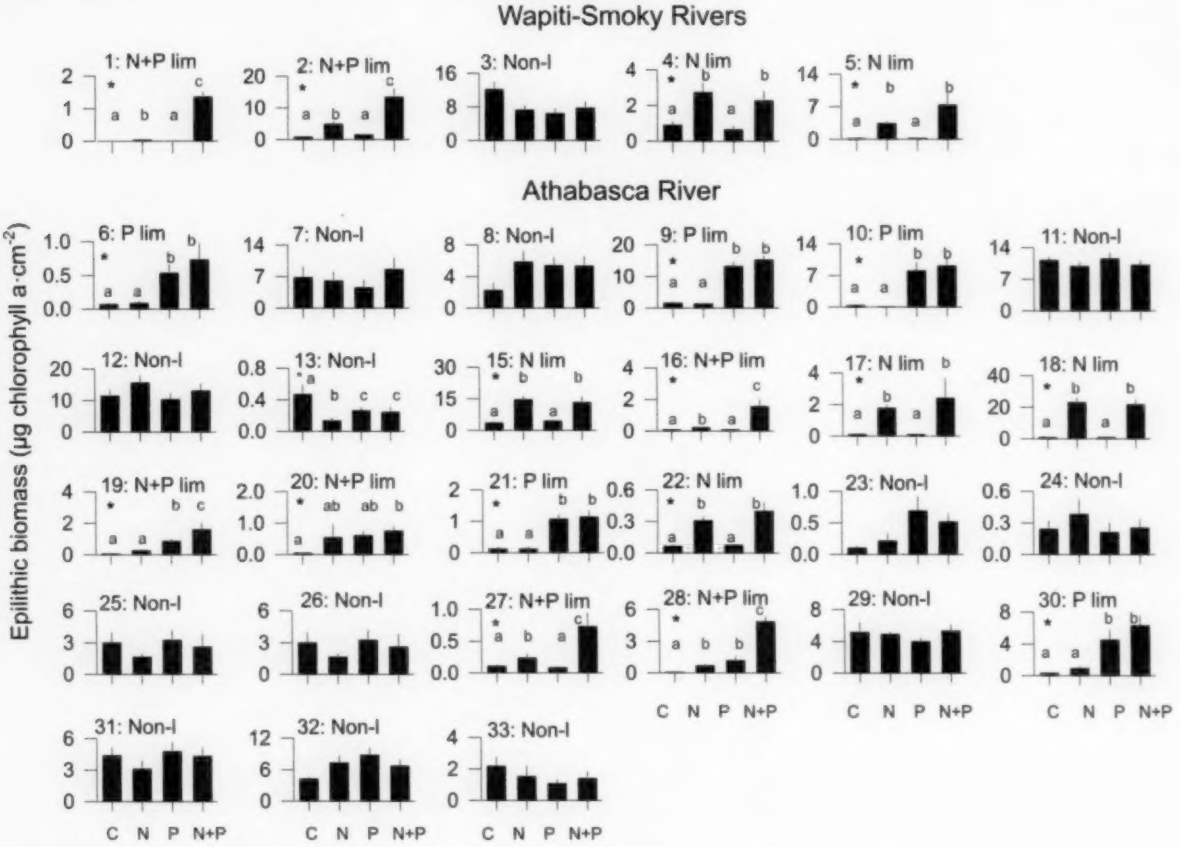
Note: —, no difference. Nutrient status of tributary sites 16 and 29 is not shown. Low numbers of study sites precluded comparisons of N and P deficiency in the Wapiti–Smoky rivers. STP, sewage treatment plant; BKPM, bleached kraft pulp mill; CTMPM, chemithermomechanical pulp mill.

Spatial patterns in nutrient limitation

In the Wapiti River, epilithic algae at the most upstream site and immediately downstream of the STP (sites 1 and 2) were N+P deplete but were N+P replete downstream of the pulp mill (site 3) (Fig. 5). The absence of a detectable effect of the Grande Prairie sewage discharge on N and P limitation may arise because these discharges are intermittent where 2 weeks of discharges are followed by a 2-week period of no releases. In the Smoky River, epilithic algae were N limited 12 and 94 km downstream (sites 4 and 5) of its confluence with the Wapiti River (Fig. 5).

Epilithic communities in the upper reaches of the Athabasca River were P limited, switched to N+P-replete conditions 2.5 and 4 km downstream of the first STP, but reverted to P limitation by 20 km downstream of the STP (site 9) (Fig. 5). The combined municipal and BKPM discharge into the Athabasca River at Hinton also resulted in a transition from P limitation (site 10) to nutrient-replete conditions (sites 11–13). At greater distances downstream of Hinton (sites 15–22), epilithic algae switched to being N and N+P limited coincident with discharges from two CTMPMs and one municipal STP. Epilithic algae were also N+P limited in the McLeod River (site 16). Accrual of algal biomass on NDS at site 14 was particularly low (overall mean $<0.1\ \mu g \cdot cm^{-2}$), likely resulting from the deployment of NDS on the south side of the river where a 4-m bank and mature riparian forest (20 m in height) severely reduced light availability. These data were excluded from analyses. Epilithic

Fig. 5. Mean (±1 SE) concentrations of Chl *a* on NDS at 32 sites in the Wapiti–Smoky and Athabasca rivers, fall 1994. Sites are numbered 1–13 and 15–33. *Significant ($p < 0.05$) ANOVA models. N lim, N limited; P lim, P limited; N+P lim, N+P limited; Non-l, nonlimited. Conclusions of N and P limitation were based on pairwise comparisons of treatment means using Bonferroni-adjusted *p* values. Histograms sharing the same letters are not significantly different.



algae in the Athabasca River 10 km upstream of the confluence of the Lesser Slave River (site 22) were N limited but were N+P replete 3 km downstream of the confluence (site 23) (Fig. 5). At further distances downstream, epilithic communities shifted from N+P replete (sites 24–26) to N+P limited (site 27). While epilithic algae upstream of Fort McMurray were N+P limited (site 28), all sites downstream of the confluence of the Clearwater River, where algae were N+P replete, and the Fort McMurray STP were also nutrient replete (i.e., sites 31–33) (Fig. 5). Our analysis of differences in nutrient limitation between sites upstream and downstream of point-source inputs showed that inputs on average altered patterns in nutrient limitation for 78 km (overall range 4–120 km) (Table 3).

Our comparison of nutrient limitation status expected from SRP and DIN concentrations and DIN:SRP molar ratios compared relatively poorly with that observed using NDS (Table 4). DIN and SRP concentrations correctly identified 17% (one of six) of the six N-limited sites, 40% (two of five) of the P-limited sites, 57% (four of seven) of the N+P-limited sites, and 36% (five of 14) of the nonlimited sites. Similarly, DIN:SRP molar ratios correctly identified 17% (one of six) of the N-limited sites, 80% (three of five) of the P-limited sites, and 29% (two of seven) of the N+P-limited sites (Table 4).

Discriminating between nutrient-limited and nutrient-nonlimited sites

Our NDS experiments identified 18 sites as being nutrient limited (five P limited, six N limited, seven N+P limited) and 14 sites as N+P replete (Fig. 5). Nutrient-replete sites had significantly (*t*-test on $\log_{10}$ transformed data, $p < 0.05$) higher combined concentrations of SRP and DIN (nutrient-replete sites: 1142 ± 720 $\mu g \cdot L^{-1}$, nutrient-limited sites: 46 ± 6 $\mu g \cdot L^{-1}$) and marginally significantly (*t* test on $\log_{10}$-transformed data, $p = 0.054$) higher seston biomass compared with nutrient-limited sites (nutrient-replete sites: 3.9 ± 1.1 $\mu g \cdot L^{-1}$, nutrient-limited sites: 1.5 ± 0.2 $\mu g \cdot L^{-1}$). Water temperature, light attenuation coefficients, and epilithic Chl *a* from upper stone surfaces did not differ significantly between nutrient-replete and nutrient-deplete sites. Stepwise discriminant function and canonical analyses showed that

Table 4. Comparison of observed and expected nutrient limitation in the Wapiti–Smoky and Athabasca rivers based on DIN and SRP concentrations and DIN:SRP molar ratios.

Site	DIN ($\mu g \cdot L^{-1}$)	SRP ($\mu g \cdot L^{-1}$)	Expected limiting nutrients	DIN:SRP molar ratio	Expected limiting nutrient	Observed nutrient limitation status
1	11	5	N	4.8	N	N+P limited
2	13	4	N+P	6.7	N	N+P limited
3	18	21	N	1.9	N	Nonlimited
4	25	9	N	5.8	N	N limited
5	21	4	N+P	11.7	N+P	N limited
6	86	2	P	111.5	P	P limited
7	77	3	P	50.3	P	Nonlimited
8	78	4	P	46.9	P	Nonlimited
9	87	9	Nonlimited	22.0	P	P limited
10	57	2	P	57.0	P	P limited
11	58	7	Nonlimited	17.9	N+P	Nonlimited
12	40	4	N+P	22.8	P	Nonlimited
13	39	7	N	11.7	N+P	Nonlimited
15	56	5	Nonlimited	27.5	P	N limited
16	22	4	N+P	12.7	N+P	N+P limited
17	44	3	N+P	28.8	P	N limited
18	76	10	Nonlimited	17.3	N+P	N limited
19	48	7	N	14.7	N+P	N+P limited
20	17	5	N	7.3	N	N+P limited
21	44	10	N	9.5	N	P limited
22	37	2	N+P	33.9	P	N limited
23	16	5	N	6.9	N	Nonlimited
24	40	5	N	15.3	N+P	Nonlimited
25	415	85	Nonlimited	10.8	N+P	Nonlimited
26	26	4	N+P	13.4	N+P	Nonlimited
27	40	3	N+P	26.3	P	N+P limited
28	32	2	N+P	35.8	P	N+P limited
29	57	13	Nonlimited	9.5	N	Nonlimited
30	28	3	N+P	21.7	P	P limited
31	5996	7	Nonlimited	1950.0	P	Nonlimited
32	8695	40	Nonlimited	480.7	P	Nonlimited
33	219	4	P	112.7	P	Nonlimited

Note: Expected nutrient limitation based on N and P water column concentrations assume DIN- and SRP-replete conditions of 55 and 5 $\mu g \cdot L^{-1}$, respectively. Observed patterns in nutrient limitation are based on results of NDS.

the combined concentration of SRP and DIN was a significant discriminator between nutrient-replete and nutrient-deplete sites and that there was a relatively strong relationship between discriminant scores and group membership ($r = 0.42$). The discriminant function had an overall classification success of 69% (22 of 32) and correctly identified all 18 of the nutrient-limited sites as being nutrient limited but only four of the 14 (29%) nutrient-nonlimited sites as being nutrient nonlimited.

Discussion

Our results from the Athabasca and Wapiti–Smoky rivers showed that epilithic biomass and nutrient status were strongly affected by anthropogenic nutrient inputs. On average, the effects of anthropogenic nutrient inputs on algal biomass were about one quarter (i.e., overall mean = 21 km) of that related to changes in N and P limitation (overall mean = 78 km) such that background nutrient concentrations, periphyton biomass, and nutrient status were typically reverted to background levels prior to the next input. However, in the middle reach of the Athabasca River, nutrient-replete conditions caused by effluent discharge at Hinton were sustained for at least 120 km.

Although many studies have evaluated the effects of nutrient enrichment on streams, most have aimed at answering mechanistic questions (such as bottom-up versus top-down control of food webs, nutrient dynamics such as spiralling, or interactions within the benthic mat assemblage), with effort largely focused on small streams with little or no anthropogenic inputs (e.g., Cattaneo et al. 1997; Kjeldsen et al. 1998). However, large rivers are the usual receiving waters for nutrient-rich discharges such as municipal sewage, certain industrial waste waters, and streams draining agricultural fields. Few studies have examined the effects of nutrient pollution on periphyton biomass and nutrient status in larger rivers (e.g., Bothwell 1992; Dube et al. 1997). In the case of the Athabasca and Wapiti–Smoky rivers, our results showed that measurements of epilithic algal biomass and nutrient status allowed identification of the extent to which upstream additions of nutrients were transmitted downstream and interacted with other point-source discharges (i.e., assessment of cumulative effects).

Despite the observation that changes in nutrient limitation were clearly associated with effluent discharges, epilithic biomass was only moderately predictable (multiple regressions, $r^2 = 0.67$) from water column N and P concentrations. Epilithic biomass was significantly ($p < 0.0001$) related to DIN and marginally significantly ($p = 0.06$) related to SRP, suggesting that epilithic biomass was determined by the availability of both N and P, a finding consistent with the results from our NDS experiment. Significant regression and correlation analyses between periphyton biomass and water column nutrient concentrations have likewise been noted for streams within regions of Denmark (Kjeldsen 1994) and Ontario (Cattaneo et al. 1997) as well as for a compilation of data from 205 sites on North American and New Zealand streams (Dodds et al. 1997). Although several studies in North America have reported no relationship between periphyton biomass and nutrient concentrations, generally due to overriding factors such as high ambient nutrient concentrations, hydrologic determinants, or top-down controls (e.g., Welch et al. 1988), most studies reported significant coefficients of determination ranging from 0.25 (Cattaneo et al. 1997) to 0.61 (Kjeldsen 1994). The similarity in the predictability of our epilithic Chl *a* model with those from other studies is surprising given that we selected sites upstream and downstream of anthropogenic inputs. Unlike other studies where remote or only moderately impacted stream reaches were investigated, we expected to see an overriding response to nutrients. However, even in controlled laboratory conditions, predictability of epilithic Chl *a* from total phosphorus (TP) is about 77% (Krewer and Holm 1982). Our observation that even dramatic changes in nutrient concentrations in intrinsically oligotrophic rivers do not produce a highly predictable response in biomass reinforces the importance of other factors in determining periphyton accrual. Some of this unexplained variation in epilithic Chl *a* may be due to herbivory, light availability, hydrologic determinants (e.g., flooding, water velocity, sediment stability), diffusion-related processes affecting the movement of nutrients from the overlying water into the epilithic mat, and nutrient cycling within the epilithic mat (e.g., Bothwell 1988; Hill et al. 1992; Steinman et al. 1995). In the case of the Athasbasca and Wapiti–Smoky rivers, unexplained variation may be caused by differences in light availability (although we quantified light levels reaching the river bottom at each site, we did not quantify the length of time that each site received direct light).

Our NDS experiment and models of epilithic biomass both indicated that availability of N as well as P controlled epilithic biomass in the Athabasca and Wapiti–Smoky rivers. Inorganic P is commonly considered to be the element most likely to limit algal production in freshwaters (e.g., Hecky and Killam 1988). Although much of the evidence supporting this claim is based on studies of phytoplankton, a number of stream studies have indicated that P limits the growth of benthic algae (e.g., Bothwell 1989; Kjeldsen et al. 1998). However, N has been identified as the limiting nutrient in some lotic ecosystems, particularly for watersheds that are rich in geologic sources of P or that are dominated by agriculture (Grimm and Fisher 1986; Bothwell 1992; Chessman et al. 1992). Regression analysis by Dodds et al. (1997) of benthic Chl *a* and nutrient concentrations for 205 sites from streams in North American and New Zealand has also shown that total nitrogen (TN) and TP are better predictors of mean and maximum biomass together than TP (or SRP) alone. In the Athabasca or Wapiti–Smoky rivers, neither the nutrient concentrations in the upper sites nor the nature of the underlying bedrock suggests that N limitation is of geologic origin. Moreover, experiments using NDS near the headwaters of the Athabasca River mainstem showed P limitation. Data are not available for the headwaters of the Wapiti River; however, given the similarity in geology and soils between the Wapiti and Athabasca rivers, the upper reach of this river is likely also P limited. Instead, N limitation in the middle reach of the Athabasca River was associated with addition of an effluent with a low TN:TP molar ratio (effluent from a CTMPM located near Whitecourt (TN:TP = 2.3)). For the Wapiti–Smoky rivers, N limitation occurred in the Smoky River after it joined with the Wapiti River where nutrient-replete conditions prevailed due to addition of kraft mill effluent. Co-limitation by N and P was common in both river systems: at the most upstream sites on the Wapiti River and at five sites in the middle to lower reaches of the Athabasca River. Although the mechanism responsible for co-limitation was not investigated, the addition of the primary limiting nutrient (P) to these inherently low-nutrient systems likely depletes the supply of the secondary limiting nutrient (N) such that both nutrients become limiting to growth. These results along with those of others (e.g., Wold and Hershey 1999) suggest that co-limitation of algae by N and P, and even N limitation, is more common in streams than previously thought and also occurs more frequently in streams than in lakes.

Our results showed that spatial patterns in nutrient limitation predicted from concentrations of DIN and SRP or DIN:SRP molar ratios compared poorly with nutrient limitation identified with NDS. On average, combined DIN and SRP concentrations correctly identified 38% (12 of 32) of N-, P-, N+P-, or nutrient-replete sites. DIN:SRP molar ratios faired no better, correctly classifying 39% (seven of 18) of all nutrient-limited sites. N:P ratios are often used as potential indicators of algal nutrient status; however, recent studies have questioned their reliability because of problems related with diffusion-related processes affecting movement of nutrients from the overlying water into the epilithic mat, nutrient cycling within the mat, species-specific N and P requirements, and N:P ratios based on different nutrient fractions (Axler et al. 1994; Wold and Hershey 1999). For example, use of DIN:SRP molar ratios is problematic because in nutrient-deficient systems, one or both nutrients may be below detection limits. The DIN:TP ratio has been argued to be a better indicator of potential nutrient limitation because organic P can become available to algae via alkaline phosphatase activity whereas particulate and organic N is more refractory (e.g., Axler et al. 1994). DIN:SRP ratios failed to identify nutrient limitation in our streams, even through N and P concentrations were often less than concentrations determined to limit periphyton growth but were still detectable (55 μg $\text{DIN}\cdot\text{L}^{-1}$ and 5 μg $\text{SRP}\cdot\text{L}^{-1}$; Grimm and Fisher 1986; Perrin et al. 1987; Bothwell 1989, 1992). In addition, N:P ratios are of little value in predicting nutrient limitation downstream of industrial or municipal effluents where high, but equivalent, loadings of both N and P may not alter N:P ratios. By comparison, our canonical discriminant analysis was moderately successful in correctly identifying river reaches as being nutrient limited or nutrient nonlimited. Although the overall classification success was 67%, the model was highly successful in classifying nutrient-deplete sites (classification success = 100%) but less successful in correctly classifying nutrient-replete sites (29%).

Our assessment of nutrient limitation in two large river systems (river distances studied: 138 km for the Wapiti–Smoky rivers and 1092 km for the Athabasca River) allowed us to assess the cumulative effects of nutrient addition and the consequences to the epilithon. Although anthropogenic discharges to the rivers certainly affected nutrient limitation, our results showed that increases in periphyton abundance and changes in N and P limitation were on average limited to 21 and 78 km, respectively. These effects typically reverted to background conditions prior to the next major nutrient input. These results demonstrate that nutrient limitation in the Wapiti–Smoky and Athabasca rivers varies spatially, largely in relation to point-source inputs. Our investigation focused on the autumn when conditions in these mountain-fed rivers are most conducive to benthic algal growth. Although nutrient limitation in these rivers may vary seasonally, physical imperatives are more likely to control benthic algal abundance in other seasons: cold temperatures and low irradiance in winter and high discharge, high sediment load, and low irradiance in spring and summer. The Wapiti–Smoky and Athabasca rivers and the larger rivers into which they drain traverse regions being newly opened for timber harvest, mining, and oil extraction. Because these rivers flow north and much of the anthropogenic activity is currently taking place near the southerly headwaters, greater effort is required to assess the effects of pollutants including N and P, not only in the immediate receiving waters but also on downstream river reaches, lakes, and coastal waters.

Acknowledgements

This work was funded by the Northern River Basins Study, National Hydrology Research Institute, and postdoctoral fellowships from the Natural Sciences and Engineering Research Council of Canada and the Sir Izaak Walton Killam Foundation (G.J.S.). We gratefully acknowledge the assistance of Jasper National Park, Weldwood of Canada Ltd. (Hinton Division), Alberta Newsprint Company, Alberta Pacific Forest Industries, and Weyerhaeuser Canada Ltd. (Grande Prairie). Nancy Scott, Shelley Manchur, Nick Scott, and Warren Zyla assisted with fieldwork and equipment preparation. Trinh Luong, Gertie Hutchinson, and Patricia Burgess processed water and epilithic samples. The manuscript was completed while G.J.S. was employed by the Alberta Research Council.

References

American Public Health Association. 1975. Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association. American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Axler, R.P., Rose, C., and Tikkanen, C.A. 1994. Phytoplankton nutrient deficiency as related to atmospheric deposition in northern Minnesota acid-sensitive lakes. Can. J. Fish. Aquat. Sci. **51**: 1281–1296.

Bierhuizen, J.F.H., and Prepas, E.E. 1985. Relationship between nutrients, dominant ions, and phytoplankton standing crop in prairie saline lakes. Can. J. Fish. Aquat. Sci. **42**: 1588–1594.

Bothwell, M.L. 1988. Growth rate responses of lotic periphyton diatoms to experimental phosphorus enrichment: the influence of temperature and light. Can. J. Fish. Aquat. Sci. **45**: 261–270.

Bothwell, M.L. 1989. Periphyton-limited growth dynamics of lotic periphyton diatom communities: areal biomass and cellular rate responses. Can. J. Fish. Aquat. Sci. **46**: 1293–1301.

Bothwell, M.L. 1992. Eutrophication of rivers by nutrients in treated kraft pulp mill effluents. Water Pollut. Res. J. Can. **27**: 477–472.

Carpenter, J.H. 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnol. Oceanogr. **25**: 672–679.

Carpenter, S.R., and Cottingham, K.L. 1997. Resilience and restoration of lakes. Conserv. Ecol. **1**(1): article 2. (Available from the Internet. URL: http://www.consecol.org/vol1/iss1/art2)

Cattaneo, A., Kerimian, T., Roberge, M., and Marty, J. 1997. Periphyton distribution and abundance on substrata of different size along a gradient of stream trophy. Hydrobiologia, **354**: 101–110.

Chambers, P.A. 1996. Nutrient enrichment in the Peace, Athabasca and Slave rivers: assessment of present conditions and future trends. Northern River Basins Study Synthesis Report 4. Northern River Basins Study, Edmonton, Alta.

Chessman, B.C., Hutton, P.E., and Burch, J.M. 1992. Limiting nutrients for periphyton growth in sub-alpine forest, agricultural, and urban streams. Freshwater Biol. **28**: 349–361.

Dodds, W.K., Smith, V.H., and Zander, B. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. Water Res. **31**: 1738–1750.

Dube, M.G., Culp, J.M., and Scrimgeour, G.J. 1997. Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent. Can. J. Fish. Aquat. Sci. **54**: 2584–2595.

Environment Canada. 1994. Hydat CD ROM version 3.0. Surface water data. Environment Canada, Water Survey of Canada, Ottawa, Ont.

Francoeur, S.N., Biggs, B.J.F., Smith, R.A., and Lowe, R.L. 1999. Nutrient limitation of algal biomass accrual in streams: seasonal patterns and a comparison of methods. J. North Am. Benthol. Soc. **18**: 242–260.

Grimm, N.B., and Fisher, S.G. 1986. Nitrogen limitation in a Sonoran desert stream. J. North Am. Benthol. Soc. **5**: 2–15.

Hecky, R.E., and Killam, P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnol. Oceanogr. **33**: 796–822.

Hill, W.R., Boston, H.L., and Steinman, A. D. 1992. Grazers and nutrients limit lotic primary productivity. Can. J. Fish. Aquat. Sci. **49**: 504–512.

Kjeldsen, K. 1994. The relationships between phosphorus and peak biomass of benthic algae in small lowland streams. Verh. Int. Ver. Limnol. **25**: 1530–1533.

Kjeldsen, K., Iversen, T.M., Thorup, J., and Winding, T. 1998. Benthic algal biomass in an unshaded first-order lowland stream: distribution and regulation. Hydrobiologia, **377**: 107–122.

Krewer, J.A., and Holm, H.W. 1982. The phosphorus – chlorophyll *a* relationship in periphytic communities in a controlled ecosystem. Hydrobiologia, **94**: 173–176.

Lohman, K., Jones, J.R., and Baysinger-Daniel, C. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. J. North Am. Benthol. Soc. **10**: 14–23.

Lohman, K., Jones, J.R., and Perkins, B.D. 1992. Effects of nutrient enrichment and flood frequency on periphyton biomass in northern Ozark streams. Can. J. Fish. Aquat. Sci. **49**: 1198–1205.

Mulholland, P.J., Marzolf, E.R., Hendricks, S.P., Wilkerson, R.V., and Baybayan, A.K. 1995. Longitudinal patterns of nutrient cycling, and periphyton characteristics in streams: a test of the upstream–downstream linkage. J. North Am. Benthol. Soc. **14**: 357–370.

Nusch, E.A. 1980. Comparison of different methods for chlorophyll and phaeopigment analysis. Ergeb. Limnol. **14**: 14–36.

Perrin, C.J., Bothwell, M.L., and Slaney, P.A. 1987. Experimental enrichment of a coastal stream in British Columbia: effects of organic and inorganic additions on autotrophic periphyton production. Can. J. Fish. Aquat. Sci. **44**: 1247–1256.

SAS Institute Inc. 1987. SAS user's guide: statistics. SAS Institute Inc., Cary, N.C.

Schantz, F., and Juon, H. 1983. Two different methods of evaluating nutrient limitations of periphyton bioassays, using water from the River Rhine and eight of its tributaries. Hydrobiologia, **102**: 187–195.

Scrimgeour, G.J., and Chambers, P.A. 1997. Development and application of a nutrient-diffusing bioassay for large rivers. Freshwater Biol. **38**: 221–231.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of freshwater. 2nd ed. Misc. Spec. Publ. Fish. Environ. Can. 26.

Steinman, A.D., Mulholland, P.J., and Beauchamp, J.J. 1995. Effects of biomass, light, and grazing on phosphorus cycling in stream periphyton communities. J. North Am. Benthol. Soc. **14**: 371–181.

Strong, W.L., and Leggat, K.R. 1992. Ecoregions of Alberta. Land Information Services Division, Alberta Forestry, Lands and Wildlife, Edmonton, Alta.

Tabachnick, B.G., and Fidell, L.S. 1983. Using multivariate statistics. Harper and Row, New York.

U.S. Environmental Protection Agency. 1996. Environmental indicators of water quality in the United States. EPA 841-R-96-002. U.S. Government Printing Office, Washington, D.C.

Welch, E.B., Jacoby, J.M., Horner, R.R., and Sealey, M.R. 1988. Nuisance biomass levels of periphytic algae in streams. Hydrobiologia, **157**: 161–168.

Wold, A.P., and Hershey, A.E. 1999. Spatial and temporal variability of nutrient limitation in 6 North Shore tributaries to Lake Superior. J. North Am. Benthol. Soc. **18**: 2–14.

Potential contact statistics for measuring scale-dependent spatial pattern and association: an example of northern cod (*Gadus morhua*) and capelin (*Mallotus villosus*)

Richard L. O'Driscoll, David C. Schneider, George A. Rose, and George R. Lilly

Abstract: Analysis of simulated data showed that potential contact statistics could be used to describe spatial pattern in sample density data. Potential contact is a new method, analogous to Ripley's *K* function for mapped point pattern analysis. Potential contact can be used to describe spatial pattern and association over a range of scales without grouping data and is robust against the presence of zeros. The statistical output is ecologically interpretable, as a measure of the degree of contact between individuals. This new technique was applied to examine changes in the spatial distribution of Atlantic cod (*Gadus morhua*) off Newfoundland, Canada, from 1985 to 1994, a period that encompassed a collapse of the cod stock. Sample data from bottom-trawl surveys indicated that cod were aggregated in patches with dimensions of 100–250 km. During the period of cod decline in the 1990s, spatial structure changed in three ways: the number of patches decreased, patch size shrank, and contact with conspecifics at small (10–20 km) scales fell. Cod were broadly associated with capelin (*Mallotus villosus*), a major prey species. Spatial distribution of capelin changed over the same time period as changes in cod distribution, and there was no evidence that contact between cod and capelin decreased.

Résumé : L'analyse de données simulées a montré que le calcul du contact potentiel pouvait servir à décrire le régime spatial dans des données sur la densité dans des échantillons. Le contact potentiel est une nouvelle méthode, analogue à la fonction *K* de Ripley utilisée pour l'analyse de voisinage. Le contact potentiel peut servir à décrire le régime spatial et l'association sur une vaste gamme d'échelles sans grouper les données, et cette méthode est robuste en présence de zéros. Le résultat statistique est interprétable sur le plan écologique, comme mesure du degré de contact entre individus. Nous avons appliqué cette nouvelle technique à l'examen des changements dans la distribution spatiale de la morue (*Gadus morhua*) au large de Terre-Neuve (Canada) de 1985 à 1994, période qui a vu un effondrement du stock de morue. Les données d'échantillonnage fournies par des relevés au chalut de fond ont indiqué que les morues se rassemblaient en bancs dont les dimensions étaient de l'ordre de 100–250 km. Pendant la période de déclin de la morue des années 1990, la structure spatiale a changé de trois façons : le nombre des bancs a baissé; la taille des bancs a rétréci; enfin, le contact avec les congénères à de petites échelles (10–20 km) a chuté. La morue est fortement associée au capelan (*Mallotus villosus*), qui est sa principale proie. La distribution spatiale du capelan a changé pendant la période où la distribution de la morue s'est modifiée, et rien n'indique que le contact entre la morue et le capelan ait baissé.

[Traduit par la Rédaction]

Introduction

The concept of scale-dependent spatial pattern is central to many aspects of fisheries ecology. The distributions of aquatic organisms are almost always patchy, and the degree of spatial heterogeneity usually varies with scale (Schneider 1994). Consequently, most fisheries ecologists are faced at some time with the problem of quantifying scale-dependent patterns. Description of observed spatial pattern over a range of scales is the important first step towards determining, understanding, and modelling the underlying pattern-generating processes (Levin 1992) and also in defining appropriate sampling strategy for hypothesis testing (Andrew and Mapstone 1987).

Methods for describing scale-dependent spatial pattern range from simple graphical representation to techniques involving extensive mathematical computation. Legendre and

Received November 30, 1999. Accepted April 14, 2000.
J15456

R.L. O'Driscoll[1] and G.A. Rose. Fisheries Conservation, Fisheries and Marine Institute, Memorial University of Newfoundland, P.O. Box 4920, St. John's, NF A1C 5R3, Canada.
D.C. Schneider. Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF A1C 5S7, Canada.
G.R. Lilly. Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, NF A1C 5X1, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: odriscol@caribou.mi.mun.ca

Fortin (1989) distinguished between point pattern analysis, where the location of all individuals is known, and surface pattern analysis, where the distribution is spatially continuous and observations are only available at discrete sample points. In general, techniques for point pattern analysis are based on the distance separation between individuals (e.g., nearest neighbour, Ripley's *K*: Ripley 1981; Cressie 1991), while techniques for surface pattern analysis are based on estimation of the lagged covariance function (e.g., spectral analysis: Platt and Denman 1975; autocorrelation: Legendre and Fortin 1989; geostatistics: Rossi et al. 1992) or grouped covariance structure (e.g., variance to mean ratio: Schneider and Piatt 1986).

Existing point pattern analysis and surface pattern analysis methods have disadvantages when applied to ecological data. It is only feasible to map the positions of all individuals in an area at small spatial scales, especially when these individuals move. As a result, most ecological applications of point pattern analysis techniques have been in studies of vegetation (e.g., Andersen 1992) or of relatively sedentary invertebrates (e.g., Muotka and Penttinen 1994) at small scales. Surface pattern analysis methods require a data set where a large number (>30) of sampling points are spaced over the entire area, preferably in a regular grid. Because many surface pattern analysis techniques are based on a measure of lagged covariance, they are sensitive to the presence of zeros and outlying values in the data set. Points with zero values will autocorrelate, overestimating covariance (Legendre and Fortin 1989), and outliers will also bias the results. Individual methods have further limitations. For example, spectral analysis may not detect nonperiodic patterns (Logerwell et al. 1998). Other surface pattern analysis methods require the data to be grouped to describe covariance at larger spatial scales. This introduces problems with defining group boundaries and reduced sample size at large scales (Malatesta et al. 1992).

In this paper, we present a new method to describe spatial structure in sampled data from continuous two-dimensional distribution surface patterns. Potential contact is a distance-based method, analogous to Ripley's *K* function for point pattern analysis. This new technique allows description of spatial pattern across a wide range of scales, is robust to the presence of zeros, and can be used in an area with irregular boundaries and sampling. An added advantage of this method is that the output is easily interpreted ecologically. Our test statistic is a direct measure of the contact of an individual with neighbouring individuals and is defined as the average number of neighbouring individuals within a distance, *t*, of any individual in the distribution. Contact is of interest in many ecological processes, including social interaction, resource utilisation, and genetic recombination. Potential contact is a generalisation of mean crowding (Lloyd 1967). However, unlike Lloyd's (1967) index of mean crowding, potential contact may be calculated at increasing spatial scales (increasing *t*) without grouping data. The concept of potential contact was introduced by Schneider et al. (1987) and described by Schneider (1994), but we are aware of only one other ecological study in which contact has been used directly as a measure of spatial structure (Bult et al. 1998). Our study differs from that of Bult et al. (1998) because we apply the method to sampled surface data rather than to a mapped point process.

We illustrate our statistical methodology using simulated data and then apply these new techniques to describe large-scale (tens to hundreds of kilometres) spatial pattern in the distribution of Atlantic cod (*Gadus morhua*) using data from bottom-trawl research surveys in Newfoundland, Canada, waters in 1985–1994. This data set provides an example of an ecological application of our techniques. Previous analysis of these and other data from this region indicates that the spatial distribution of cod changed during the 1990s (review in Rose et al. 2000). These changes, notably a southward contraction in the area occupied by the cod stock, may have made the fish more vulnerable to overfishing (Rose et al. 2000), leading to the collapse and closure of the northern cod fishery off Newfoundland in 1992. We were interested in how the spatial pattern of the cod, and specifically their potential contact with other cod, changed during this period of stock collapse. Maintenance of potential contact as cod numbers declined could account for the failure of catch-per-unit-effort indices to track stock size (Rose and Kulka 1999).

In addition to describing spatial pattern and aggregation of individuals, Ripley's *K* may be used to analyse bivariate patterns and association in point patterns (Lotwick and Silverman 1982). Similarly, our related measure of potential contact may be used to analyse interspecific associations in sampled data from continuous surface patterns. We present examples based on simulated data and then use the bottom-trawl data set to examine scale-dependent spatial association between cod and capelin (*Mallotus villosus*) off Newfoundland in 1985–1994. Capelin are the major prey of cod in this region (review in Lilly 1987).

Methods

Statistical methods

We define potential contact as the number of neighbours potentially encountered by an individual within an ambit of given size. For sample density data from a continuous surface pattern, potential contact may be expressed as

$$(1) \qquad \mathrm{PC}(t) = \pi t^2 E[d(t)].$$

The $E[d(t)]$ is the expected density of neighbours within a circular ambit of radius t and πt^2 is the area of ambit radius t. The $E[d(t)]$ is calculated empirically as a weighted average. For each sample point $i = 1,2,...,n$, density estimates (d_j) from all other sample points within distance t (indicator function $I_{ij} = 1$ where distance $ij < t$ and 0 otherwise) are averaged and scaled by the density at point i (d_i). The result is then summed and divided by the sum of the point density estimates to estimate the expected density of neighbours within distance t:

$$(2) \qquad E[d(t)] = \frac{\sum_{i=1}^{n} d_i(\overline{d_{ij}})}{\sum_{i=1}^{n} d_i}$$

where

$$(3) \qquad (\overline{d_{ij}}) = \frac{\sum_{j=1}^{n} (I_{ij}) d_j}{\sum_{j=1}^{n} I_{ij}}.$$

Ambit size (and spatial scale) is increased by incrementally increasing ambit radius t.

The PC(t) is analogous to $E[N(t)]$, the expectation term of Ripley's K function for a spatial point pattern (Ripley 1981). The $E[N(t)]$ is the Lagrangian equivalent of potential contact, where cooccurrence is measured as a function of distance from an individual and is not constrained by sampling points.

There is a sampling uncertainty associated with PC(t) because $E[d(t)]$ is calculated from sample estimates of density. We estimated sampling uncertainty using a jackknife approach (Manly 1997). Individual sample points ($i = 1,2,...,n$) were removed sequentially and PC(t) was calculated for each of the reduced data sets. The variability among the n jackknifed values of PC(t) was used to estimate the standard error (Manly 1997).

To test whether an observed spatial distribution was significantly different from a random arrangement, we randomised the density pattern by randomly reallocating increments of density to the sample points. The increment of density chosen was the minimum resolution of the data. For example, if density was measured to two decimal places, we randomly reallocated density values of 0.01 until the sum of the densities across all sample points was equal to the sum of the densities in the original pattern. This randomisation method is equivalent to randomly reallocating individuals in a spatial point pattern. The randomisation process was repeated 100 times and PC(t) was calculated for each realisation. From these realisations, we calculated the average potential contact for a random arrangement, $\mathrm{PC}(t)_{\mathrm{ran}}$, and the values of potential contact equivalent to the 5th percentile, $\mathrm{PC}(t)_5$, and the 95th percentile, $\mathrm{PC}(t)_{95}$.

We express our results as the "extra contact" at distance t, XC(t):

$$(4) \qquad \mathrm{XC}(t) = \mathrm{PC}(t) - \mathrm{PC}(t)_{\mathrm{ran}}.$$

The XC(t) is the average number of "extra" neighbours within distance t. The extra neighbours are those that are not expected if the individuals were distributed randomly throughout the study area. The XC(t) is analogous to $L(t) = \sqrt{(K(t)/\pi)} - t$, commonly used as a test statistic in applications of Ripley's K function to spatial point data (Cressie 1991). The XC(t) has an expected value of zero under a null hypothesis of complete spatial randomness. When individuals are aggregated, $\mathrm{XC}(t) > 0$. The upper and lower bounds for a random spatial distribution are given by

$$(5) \qquad \mathrm{XC}(t)_{95} = \mathrm{PC}(t)_{95} - \mathrm{PC}(t)_{\mathrm{ran}}.$$

$$(6) \qquad \mathrm{XC}(t)_5 = \mathrm{PC}(t)_5 - \mathrm{PC}(t)_{\mathrm{ran}}.$$

The $\mathrm{XC}(t)_{95}$ represents the significance level of $p = 0.05$ for a one-sided test or $p = 0.10$ for a two-sided test. Because we were not considering the alternative hypothesis of uniformity, when $\mathrm{XC}(t) > \mathrm{XC}(t)_{95}$, the aggregation was considered statistically significant. No correction for edge bias (Haase 1995) was necessary in our analysis because the randomisation scheme used to generate the null model and calculate XC(t) used the same sampling points and makes the same assumptions about the distribution of organisms outside the study area as the data.

We used a Matlab® routine to calculate XC(t), $\mathrm{XC}(t)_5$, and $\mathrm{XC}(t)_{95}$ at distances $t = s, 2s, 3s,, T$, where s is the sampling scale, related to the separation between adjacent sampling points, and T is the maximum separation between sampling points. The Matlab® code is available on request from the first author. Results are presented as plots of XC(t) as a function of t. Such plots allow comparison of association over a range of scales (Haase 1995). In general, interpretation should be limited to values of $t < T/2$ (Cressie 1991). The XC(t) drops back to zero as t approaches T because all data become encompassed by the calculation. We do not include estimates of sampling uncertainty for XC(t) values themselves. Description of spatial pattern was based on relative levels of contact from observed and randomised arrangements of fish which were not affected by sampling uncertainty.

Bivariate analysis

Our measure of contact may also be used to examine associations between different species. By analogy with eqs. 2 and 3, the expected density of type B individuals within distance t of a type A individual, $E[b(t)]$, is

$$(7) \qquad E[b(t)] = \frac{\sum_{i=1}^{n} a_i(\overline{b_{ij}})}{\sum_{i=1}^{n} a_i}.$$

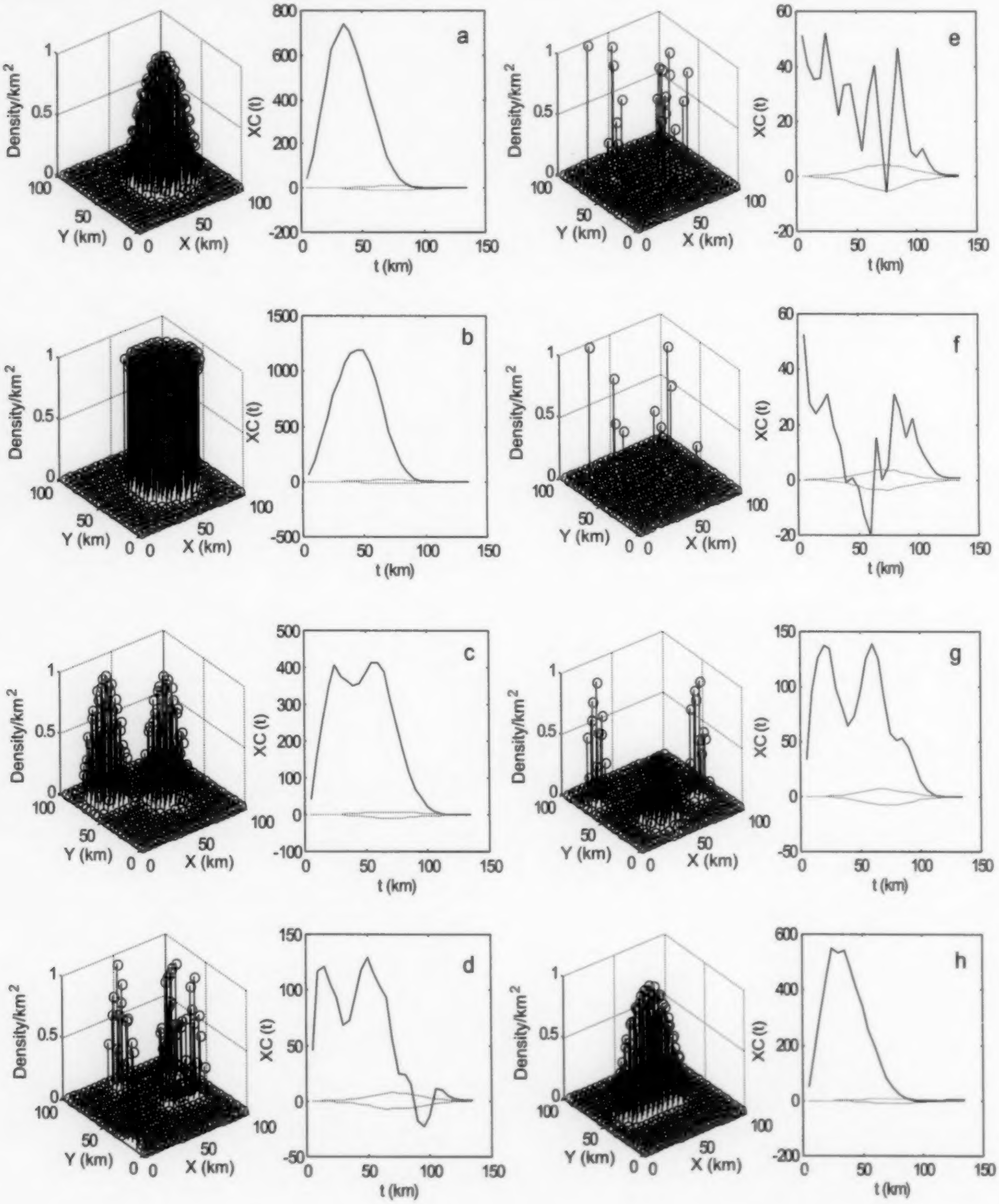
The a_i and b_j are the measured densities of type A and type B individuals at sample point i and j, respectively. The PC(t) and XC(t) are calculated from eqs. 1 and 4 as above. Bivariate XC(t) is a measure of the extra contact with type B individuals within distance t of a type A individual. The measure is easily interpreted. A value of zero at distance t indicates no association at that scale, values greater than zero indicate positive association or correlation, and values less than zero indicate negative association or segregation. The results are displayed as plots of XC(t) as a function of t.

A randomisation procedure was again used to assess whether association was statistically significant, but it was more difficult to choose a null model. To be a valid test of association, the null (random) patterns of both type A and type B individuals should retain some of the spatial properties of the observed (data) patterns while randomising their relative positions (Roxburgh and Chesson 1998). This is achieved in part by only randomising the distribution of one group. In this paper, we compare four alternative null models based on different methods of randomisation: (*i*) random reallocation of density increments (individuals) as used in univariate analysis, (*ii*) random rearrangement of density values between sampling points, (*iii*) random toroidal shift of density values, and (*iv*) random repositioning of clump centres. The first two models change the second-order properties of the spatial distribution. However, both these methods have been used in the literature (Bult et al. 1998; O'Driscoll 1998). The toroidal shift method suggested by Lotwick and Silverman (1982) has been the most commonly used null model in analysis of bivariate point processes (e.g., Andersen 1992; Muotka and Penttinen 1994). Random repositioning of clump centres is a new method suggested by Coomes et al. (1999).

Simulated data

Continuous surface spatial patterns were generated in Matlab®. Univariate patterns were based on a random arrangement of groups of individuals (patches) within a 100 × 100 km area. The size and number of groups were set and the routine positioned the patches at random within the area. Individual patches were usually circular (Fig. 1). In most cases, density varied across the patch in a sinusoidal fashion, with a peak density of 1 individual/km^2 at the centre (Fig. 1). Densities outside the patches were zero. All patterns were sampled using a regular 20 × 20 grid of stations 5 km apart to obtain point estimates of density. We then investigated the influence of patch radius, number of patches, and density variation within a patch on contact XC(t) across a range of spatial scales, t = 5, 10, 15,...,140 km.

Fig. 1. Description of scale-dependent spatial pattern in simulated data. Plots on the left show a 20 × 20 grid of discrete samples from the simulated distribution. Plots on the right are of extra contact with neighbours as a function of scale (see text for details). Dotted lines are the 5 and 95% bounds for a random pattern generated from 100 randomisations of density increments. Values of XC(t) above the upper confidence interval were considered statistically significant (nonrandom aggregation). Simulated patterns: (*a*) single circular patch of radius 30 km with sinusoidal density variation within the patch; (*b*) single circular patch of radius 30 km with uniform density within the patch; (*c*) two circular patches of radius 20 km; (*d*) five circular patches of radius 10 km; (*e*) 10 circular patches of radius 5 km; (*f*) 20 circular patches of radius 2 km; (*g*) two circular patches of radius 10 km and a single circular patch of radius 20 km; (*h*) anisotropic patch with dimensions 60 × 20 km.



Bivariate patterns were simulated by generating a second spatial pattern, where the centres of patches of type B individuals were separated by a known distance from the centres of patches of type A individuals. We used bivariate simulations to illustrate the effect of varying separation on the observed scale of association and also to investigate the choice of null model on the significance test.

Bottom-trawl survey data

Atlantic cod and capelin were caught during annual fall (October–December) bottom-trawl surveys off Newfoundland from 1985 to 1994. Trawl surveys were based on a stratified random design giving a total of between 357 and 543 trawl stations each year (Table 1). The survey design ensured relatively uniform spatial coverage with separation between adjacent stations of 1–60 km (mean separation 14.6 km, Table 1). Tows were made using an Engel-145 high-rise otter trawl with a mesh liner of 29 mm towed for 30 min at 3.5 kn from either the 74-m *Gadus Atlantica* or the 50-m *Wilfred Templeman*. The constant wing spread value used for this trawl was 45 ft (13.5 m), meaning that the total bottom area covered in a 30-min tow was 0.044 km^2. Fishing sets covered the full 24-h period. Numbers of cod and mass (kilograms) of capelin in each catch were scaled by tow area and expressed per square kilometre with no correction for catchability. This gave point estimates of cod and capelin density at the position of the tow. We used these data to calculate the potential contact of cod with other cod and of cod with capelin at scales of t = 10, 20, 30,...,1260 km.

Results

Simulation results: description of spatial pattern

Plots of XC(t) as a function of t can be used to describe characteristics of a spatial pattern (Fig. 1). A single patch gives a single peak in XC(t) (Fig. 1*a*). The value of t at which this peak occurs is a measure of the patch extent. The patch extent is a function of both the patch dimensions and the arrangement of densities within the patch. Patterns in Figs. 1*a* and 1*b* have the same patch radius (30 km) but different surfaces. In Fig. 1*a*, peak XC(t) occurs at t = 35 km, while in Fig. 1*b*, peak XC(t) is at t = 45 km. The value of XC(t) is a measure of the intensity of crowding within a patch. For example, the peak XC(t = 35) in Fig. 1*a* indicates that each individual has 737 more neighbouring individuals within 35 km than would be expected in a random arrangement. The equivalent XC(t = 35) in Fig. 1*b* is higher (1076) because density does not decline away from the centre of the patch.

When there are two or more patches in the pattern, the shape of the plot of XC(t) as a function of t reflects both the individual patch characteristics and the separation between patches (Figs. 1*c*–1*g*). The first peak in XC(t) describes the patch characteristics. The first peak was defined as the first value of t at which XC(t) was higher than the values at the three succeeding values of t (i.e., XC(t) > XC($t + s$), XC($t + 2s$), XC($t + 3s$), where s is the increment between t values). This criterion was used to avoid labelling small jumps in contact as peaks. In Figs. 1*c*–1*f*, the value of t corresponding to the first peak in XC(t) (patch extent) occurs at 25, 15, 5, and 5 km, respectively, corresponding to simulated patch radii of 20, 10, 5, and 2 km, respectively. Intensity of crowding (peak height) also decreases in Figs. 1*c*–1*f* because there are fewer individuals in the smaller patches. Second and higher peaks in XC(t) correspond to the separation between patches. For example, in Fig. 1*c*, the second peak at XC(t = 55 km) corresponds to a distance separation between the centres of the two patches of 45 km. As more patches are added, interpretation becomes more difficult (Figs. 1*d*–*f*) due to constructive and destructive interference.

Table 1. Arrangement of bottom-trawl stations during fall surveys, 1985–1994.

Year	Number of stations	Station separation (km)		
		Minimum	Maximum	Mean
1985	543	2.74	34.68	13.40
1986	357	4.48	42.06	16.72
1987	453	3.34	37.24	15.02
1988	428	2.80	40.57	14.82
1989	450	2.18	43.62	14.54
1990	404	1.01	48.68	16.89
1991	532	1.61	48.27	13.37
1992	534	0.57	58.46	12.59
1993	416	1.90	56.97	14.58
1994	455	1.95	52.49	14.31

When patches of different sizes are present, patch extent provides a measure of the weighted average of patch size. In Fig 1*g*, two patches of radius 10 km and one patch of 20 km were sampled. Peak density in the larger patch was reduced, so total number of individuals (obtained by integrating density over the patch surface) was equivalent to the number of individuals in the two smaller patches. Patch extent, as determined by the first peak in XC(t), was at t = 20 km (Fig. 1*g*), which is intermediate between patch extent determined for patches with radii of 10 km (extent = 15 km, Fig. 1*d*) and 20 km (extent = 25 km, Fig. 1*c*).

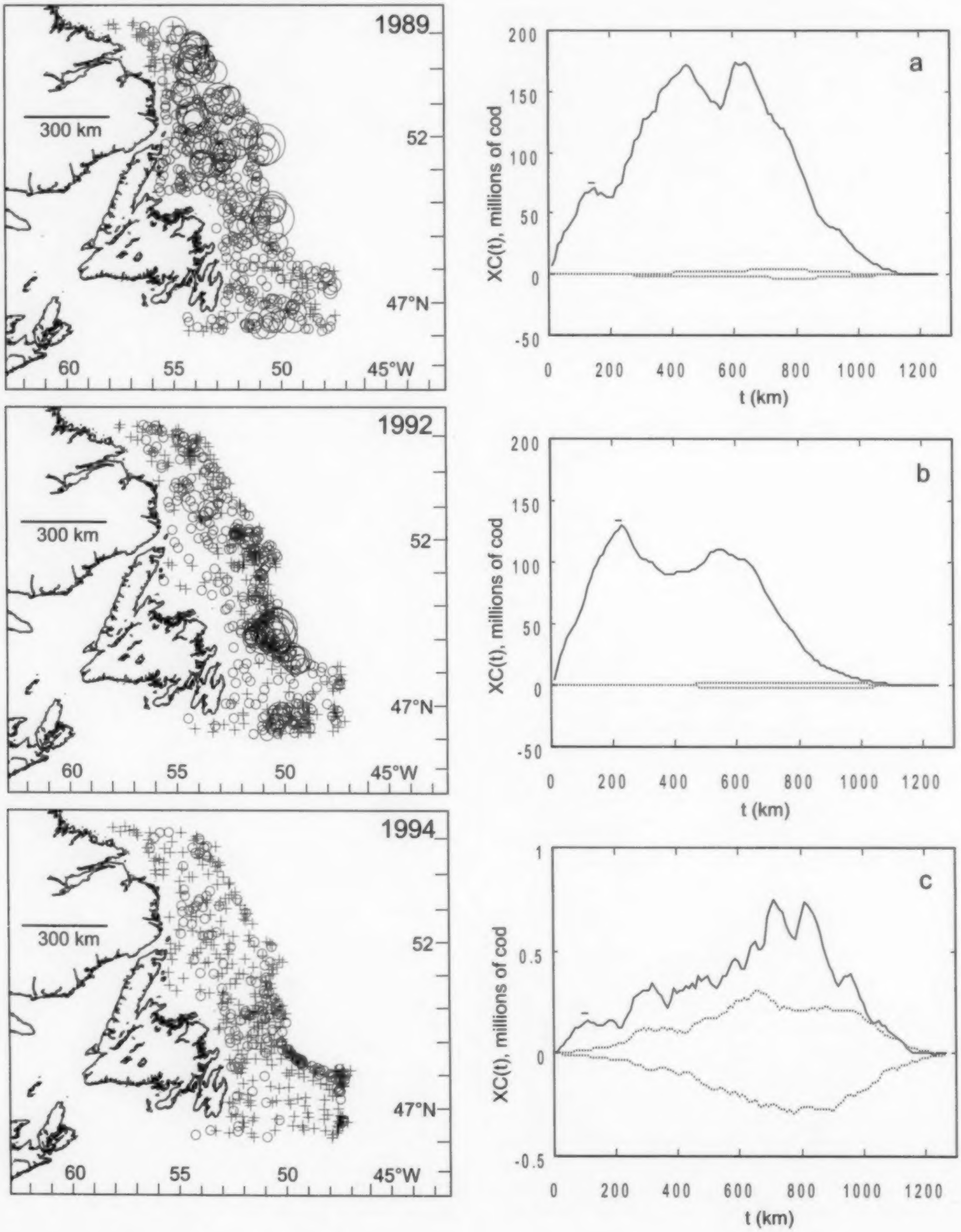
Our method assumes that patches are isotropic. When an anisotropic patch (e.g., Fig. 1*h*) was sampled, patch extent was a measure of the size of an equivalent isotropic patch. For example, the patch in Fig. 1*h* had overall dimensions of 60 × 20 km. The measured patch extent was 25 km, equivalent to the patch extent obtained from a circular patch with similar mean dimensions (radius 20 km, Fig. 1*c*).

In all these patterns, XC(t) was significantly greater than zero for most values of t. The simulated patchy arrangement of individuals meant that contact with neighbours was significantly higher than for a random arrangement, over a wide range of scales.

Description of spatial pattern in Atlantic cod distribution off Newfoundland

Spatial distributions of cod from bottom-trawl catches were explored initially using expanding symbol plots (Fig. 2). Plots of XC(t) as a function of t (Fig. 2) provided a statistical description of the spatial pattern in cod data. We set the sampling scale at 10 km, which was slightly less than average separation between adjacent stations (Table 1). In eight of 10 years between 1985 and 1994, the measured patch extent of cod was between 100 and 250 km (Fig. 3*a*). This result suggests that cod were characteristically aggregated in patches at spatial scales of hundreds of kilometres at the time of the survey. Intensity of crowding (Fig. 3*b*) was related to patch extent (Fig. 3*a*), indicating that there were more extra neighbours in larger patches.

Fig. 2. Description of spatial pattern in Atlantic cod distributions from bottom-trawl data in (*a*) 1989, (*b*) 1992, and (*c*) 1994. Maps on left are expanding symbol plots of cod catch. Circle diameters are proportional to $\log_{10}$ number of cod per standard 30-min tow. A cross indicates that no cod were captured. Plots on the right are of extra contact as a function of scale. Dotted lines are the 5 and 95% bounds for a random arrangement of cod generated from 100 randomisations of density increments. Horizontal bars indicate the first peaks that were used as a measure of patch extent.



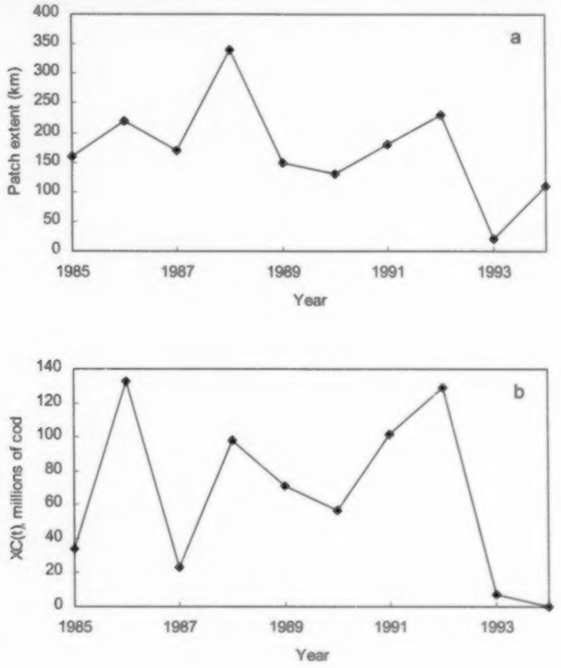


Fig. 3. Changes in (*a*) patch extent and (*b*) extra contact with neighbours at the scale of the patch extent for Atlantic cod distributions, 1985–1994.

As well as exploring patch characteristics using plots of XC(t) as a function of t, it is useful ecologically to examine values of potential contact PC(t) directly. Because extra contact is the difference between observed and expected contact at scale t (eq. 4), XC(t) is a measure of patchiness of the distribution, independent of mean density. The decline in overall density of cod off Newfoundland in the 1990s has been well documented (e.g., Hutchings 1996) and is clearly seen in a plot of mean density of cod from bottom trawl catches from 1985 to 1994 (Fig. 4*a*). Potential contact PC(t) increases with increasing ambit t as a function of both patchiness and density. If we can estimate the ambit size of the organism being studied, we can test how changes in density (Fig. 4*a*) and in patchiness (Fig. 3) combine to influence contact with conspecifics. Little is known about the ambit of cod, but mean swimming speeds during the spring migration from 1990 to 1992 were between 6.6 and 24 km/day, with a peak of 59 km/day (Rose et al. 1995). Figure 4*b* compares potential contact between cod at ambits of t = 10, 20, and 60 km from 1985 to 1994. Absolute values of PC(t) need to be treated with caution because our calculation of contact (eq. 1) is based on the assumption that similar densities of fish were present outside the study area. This is clearly not the case because the survey area is bounded to the west by land and to the east by very deep water, which is not suitable habitat for cod (Fig. 2). As a result, PC(t) will tend to overestimate contact at larger values of t. At small scales (Fig. 4*b*), almost all ambits are entirely within the surveyed area, so contact can be used as a measure of number of neighbours.

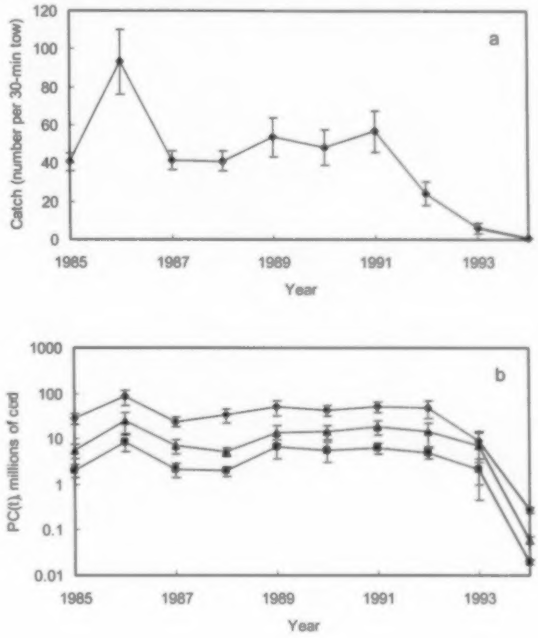
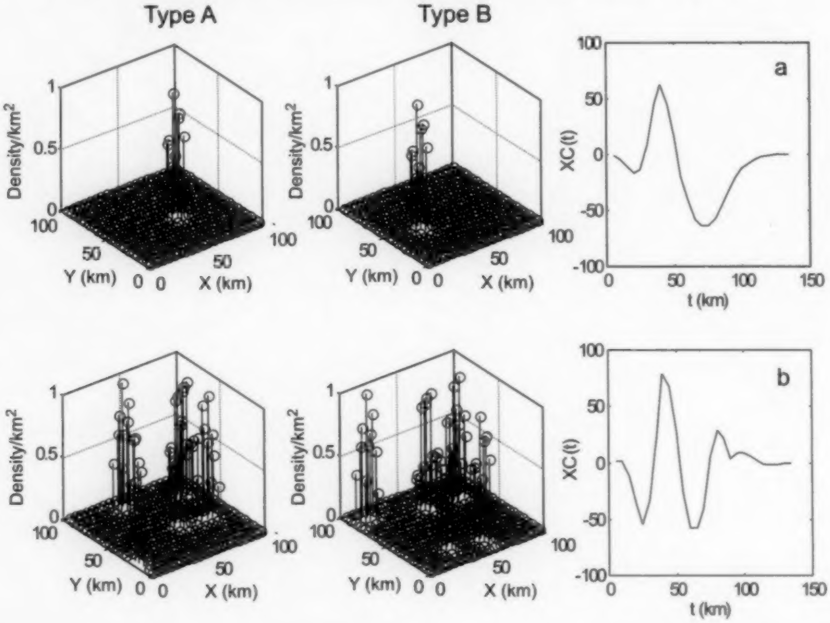


Fig. 4. Changes in (*a*) Atlantic cod catch and (*b*) potential contact with neighbouring cod at the scale of three proposed cod ambits (10 km (squares), 20 km (triangles), and 60 km (diamonds)) from bottom trawl surveys, 1985–1994. Plots show mean ± SE. Error bars around values of PC(t) are from jackknifing of the data and reflect sampling uncertainty.

Changes in spatial distribution appeared to occur sequentially as number of cod declined. In the 1980s, there were several areas with consistently high densities of cod in fall, as previously described by Lilly (1994) and Hutchings (1996). These included patches in the northern (54–55°N, 54–56°W), central (51–52°N, 50–52°W), eastern (49–50°N, 49–51°W), and southern (46–47°N, 49–51°W) parts of the surveyed region (Fig. 2*a*). Patch extent was similar in all areas (hundreds of kilometres), and plots of XC(t) as a function of scale in the 1980s were complex, with multiple peaks at large scales reflecting separation between these areas of high density (Fig. 2*a*). Potential contact (Fig. 4*b*) and intensity of crowding (Fig. 3*b*) were highest in 1986, when catches of cod (Fig. 4*a*) were unusually large. The first noticeable change in spatial structure in the 1990s was a reduction in the number of areas with high densities of cod. In 1991, there was no patch in the north, and by 1992, only patches in the eastern and southern parts of the survey region remained (Fig. 2*b*). Plots of XC(t) became less complex, with fewer peaks and less structure at larger scales (Fig. 2*b*). Patch extent (Fig. 3) and small-scale potential contact (Fig. 4*b*) were similar because the remaining patches had spatial structure similar to that of patches in the 1980s. As numbers of cod continued to decline, patch size shrank, with a dramatic decrease in our measures of patch extent (Fig. 3*a*) and individuals within a patch (Fig. 3*b*) in 1993. Potential contact with neighbours at small scales also decreased in 1993 (Fig. 4*b*), but contact with neighbours at

Fig. 5. Scale-dependent association from simulated data. Plots to left and in the centre show sampled spatial distributions of type A (left) and type B (centre) individuals. Samples are taken in a 20 × 20 grid. Plots on the right are of extra contact between type A and type B individuals as a function of scale. Simulated patterns: (*a*) single circular patches of type A and type B individuals, patches each of radius 10 km, separation between centres of type A and type B patches 30 km; (*b*) five circular patches each of type A and type B individuals, patches all of radius 10 km, separation between centres of type A and type B patches 30 km.



ambits of 10 and 20 km was still similar to that observed in the 1980s (Fig. 4*b*). The final change in spatial structure was evident in 1994, when numbers of cod had declined to very low levels (Fig. 4*a*) and potential contact with neighbours fell by two orders of magnitude (Fig. 4*b*). There were no large patches of cod, and the plot of XC(t) showed little major structure (Fig. 2*c*).

Simulation results: bivariate analysis

Contact statistics can be used to describe spatial association across a range of scales. For a simple example, consider a single patch of type A individuals separated by a known distance from a single patch of type B individuals (Fig. 5*a*). Association between the two groups is scale dependent. At scales less than the separation between groups, association is negative, indicating that there are fewer individuals of type B surrounding a type A individual than there would be if type B individuals were arranged randomly. At scales close to the separation between groups (30 km between patch centres in Fig. 5*a*), the association between groups is positive, indicating that there are more type B individuals than expected within distance t of a type A individual. At larger scales, the association becomes negative again (Fig. 5*a*). The value of t at which XC(t) is maximal can be considered as the scale of maximum association. This is the scale at which each type A individual has the most extra type B individuals surrounding it. The value of XC(t) is a measure of the intensity of the association. In Fig. 5*a*, the scale of maximum association is 40 km. Each type A individual had 62 more type B individuals within an ambit of 40 km than would be expected in a random arrangement. A more complex arrangement, where there are multiple patches of both type A and type B individuals, is illustrated in Fig. 5*b*. The resulting plot of XC(t) again reveals scale-dependent association with maximum association at a scale of 40 km.

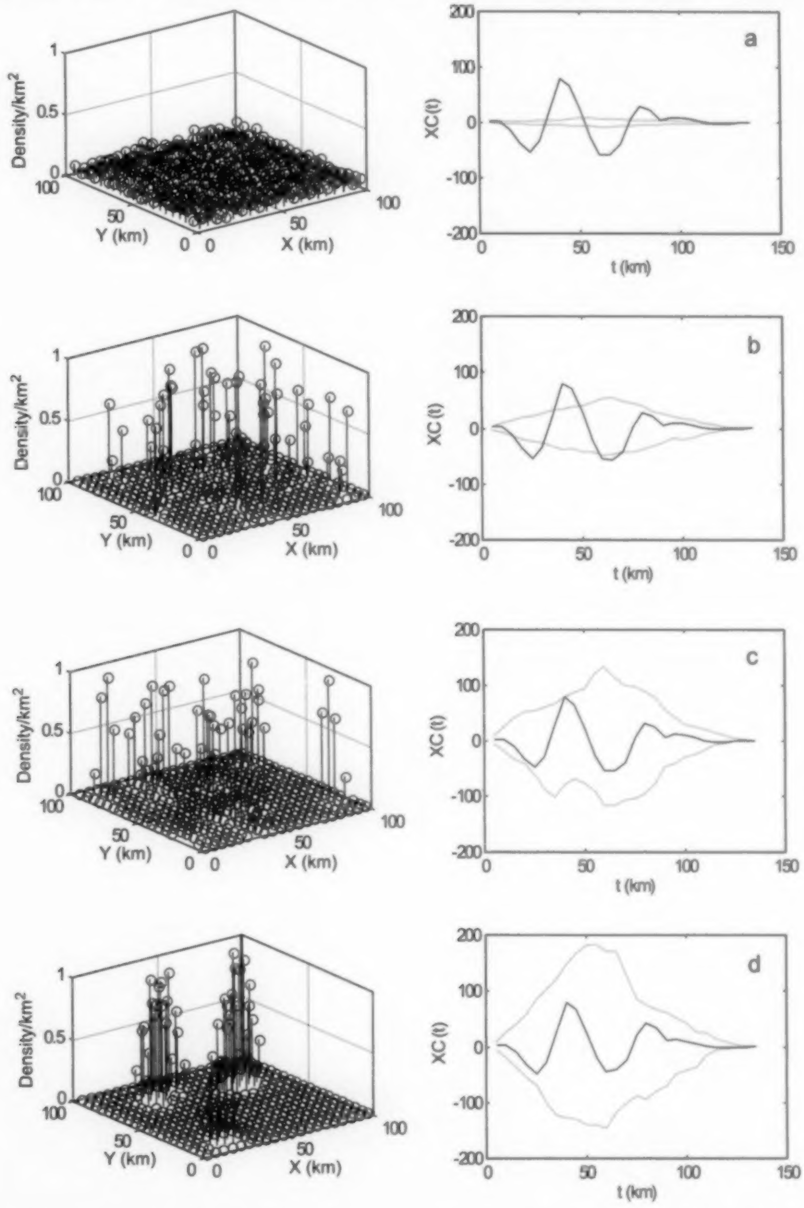
Effect of null model choice on significance of association

The statistical significance of the bivariate associations measured in Fig. 5 was highly dependent on the choice of null random model. Figure 6 shows plots of XC(t) as a function of t calculated from the distribution in Fig. 5*b* with four different null models. It is important to note that choice of null model does not influence the shape of the plot of XC(t) or affect the scale of maximum association or its intensity (Fig. 6). However, maximum association (t = 40 km) was only considered significant using two of the four null models (random individuals and random values). The two models (random shift and random clump) that preserve the second-order spatial distribution of type B individuals indicated no significant association between type A and type B individuals at any scale (Figs. 6*c* and 6*d*).

Spatial association between cod and capelin prey off Newfoundland

Spatial association between cod and capelin off Newfoundland was scale dependent. Plots of extra contact between cod and capelin from bottom trawl data are shown in Fig. 7. The procedure to calculate PC$(t)_{\mathrm{ran}}$ and confidence intervals was based on random rearrangement of cod density

Fig. 6. Effect of null model choice on significance of bivariate association. Plots on the right show scale-dependent association calculated for the simulated data in Fig. 5*b* with four different choices of random null model: (*a*) random rearrangement of increments of density (individuals); (*b*) random arrangement of observed density values; (*c*) random toroidal shift of spatial distribution; (*d*) random repositioning of patches. Dotted lines are the 5 and 95% bounds for each of the random null models generated from 100 randomisations. Plots on the left show examples of random distributions of type B individuals obtained using each of the null models.

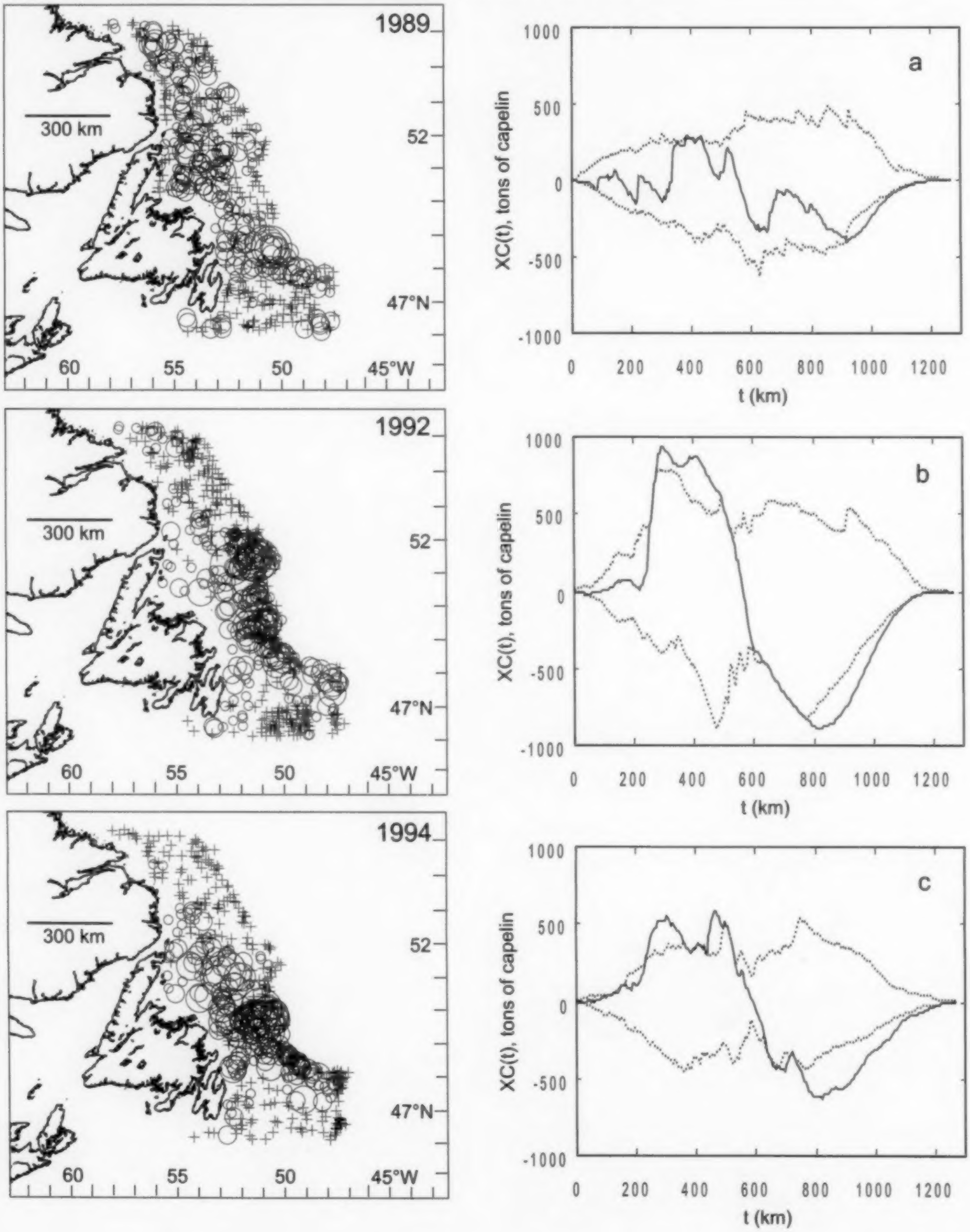


values (the second null model presented in the Methods section). Typically, there was no association between cod and capelin at scales less than 100 km, positive association at scales from 100 to 600 km, and negative association at larger scales (Fig. 7). From 1985 to 1994, scale of maximum association occurred at scales between 260 km (1990) and 920 km (1988). Cod and capelin were associated more closely from 1989 to 1994 than previously (Fig. 8*a*). Potential contact PC(t) with capelin for cod ambits of t = 10, 20, and 60 km was related to mean catch of capelin and showed no trend from 1985 to 1994 (Fig. 8*b*).

Discussion

Contact statistics provide a novel method of describing scale-dependent spatial pattern and assessing scale-

Fig. 7. Description of spatial association between Atlantic cod and capelin distributions from bottom-trawl data in (*a*) 1989, (*b*) 1992, and (*c*) 1994. Maps on left are expanding symbol plots of capelin catch. Circle diameters are proportional to $\log_{10}$ mass of capelin in kilograms per standard 30-min tow. A cross indicates that no capelin were captured. Cod distribution for the corresponding years is shown in Fig. 2. Plots on the right are of association as a function of scale. Dotted lines are the 5 and 95% bounds of spatial association for a random arrangement of cod generated from 100 randomisations of cod density values.



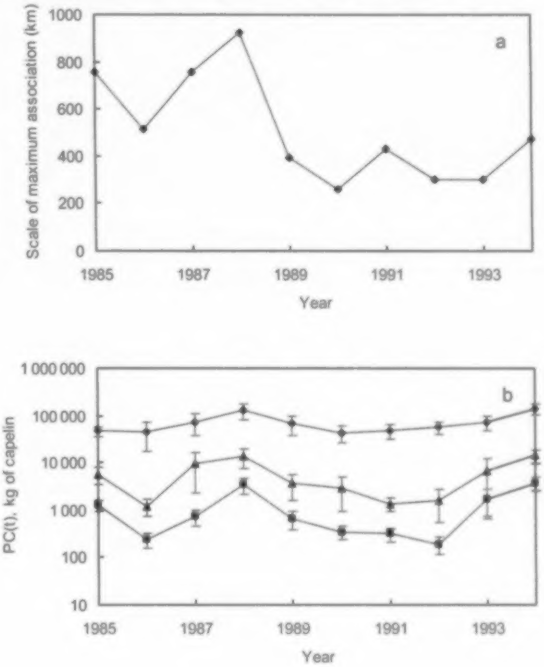
dependent spatial association. The results are easily interpreted ecologically because the output is a measure of the average number of type B individuals within a given ambit of a type A individual (Schneider 1994). Type B "individuals" could be conspecific neighbours, prey, competitors, or even abiotic features such as habitat characteristics (Bult et al. 1998). Contact may be used directly, as in this paper, to assess the potential frequency of social interactions, degree of competition, or encounters with prey. It could also be used as an input into dynamic models of social behaviour or foraging. Plots of contact as a function of ambit size provide information about spatial structure over a range of scales and may help identify relevant scales for further study.

Our statistical methods share many similarities with second-order point pattern analysis techniques, such as Ripley's *K* function (Ripley 1981), but can be applied to sampled data from a continuous surface pattern as long as the sampling scale is smaller than the spatial scales of interest. Unlike alternative covariance-based measures such as geostatistics, contact statistics are robust against the presence of zero data values. Because contact at increasing spatial scale is calculated by incrementally increasing the ambit around each sampled point, problems with grouping data and decreased sample size at larger scales (e.g., Malatesta et al. 1992) are reduced. The major disadvantage of these methods is that analysis is computationally intensive, particularly when there are a large number of sample points. Analyses in this paper took from 4 to 24 h to run on a Pentium® II 450-MHz personal computer. Computing time may be reduced for exploratory analysis by decreasing the number of randomisations or by decreasing the number or range of spatial scales (values of t) over which contact is assessed.

Sampling design is an important consideration when calculating contact statistics, as it is for any statistical method that relies on sample data (e.g., Andrew and Mapstone 1987). The separation between adjacent sampling points determines the minimum spatial scale at which contact can be assessed and also the precision with which we can describe patch extent. Patch extents measured from Atlantic cod distribution data (20–340 km) were much greater than the distance between trawl stations and the number of samples was large, so the random sample design of bottom-trawl stations was sufficient to describe spatial pattern.

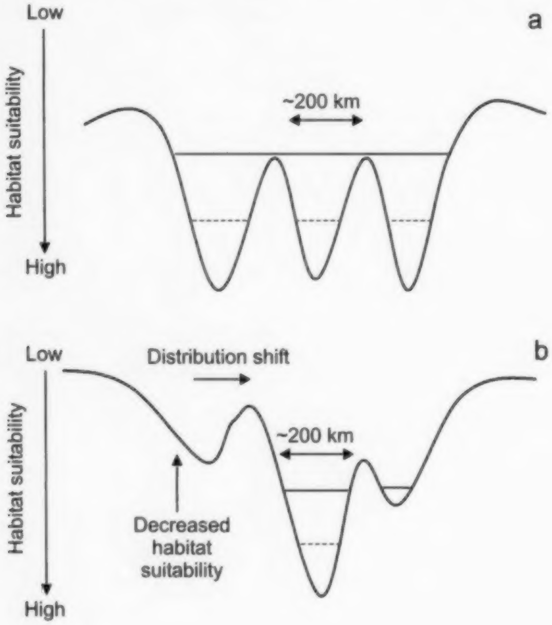
Our measurements of cod patch extent were similar to those determined by Warren (1997) based on variogram analysis of the trawl data from 1985 to 1992. Warren's (1997) analysis divided the surveyed area into three subareas based on arbitrary Northwest Atlantic Fishery Organisation management divisions. To avoid problems with zeros, Warren (1997) first reduced trawl catches to an indicator variable (presence–absence) or to the nonzero component and then fitted variogram models. The range of the variogram may be considered as a measure of patch dimensions (Rossi et al. 1992). Observed ranges for variograms fitted to the presence–absence data in the three areas were between 30 and 330 km (Warren 1997), which were similar to the patch extents that we measured (20–340 km). However, there was no significant correlation at $p = 0.05$ between our measure of patch extent over the entire survey area and Warren's (1997) estimates of variogram range in the northern ($r = 0.11$, $n = 8$), central ($r = 0.72$, $n = 6$), or southern ($r = -0.31$, $n = 8$) subareas between 1985 and 1992. Part of the reason for this was that cod patches tended to straddle the arbitrary boundaries between Northwest Atlantic Fishery Organisation divisions. We believe that our methods provide a better overall measure of spatial structure in the cod data than the analysis of Warren (1997) because we analysed the entire data set and did not need to reduce the data to avoid zero counts. Our results are also more easily interpreted from the standpoint of cod distribution ecology.

Fig. 8. Changes in (*a*) spatial scale of maximum association between Atlantic cod and capelin and (*b*) potential contact with capelin at the scale of three proposed cod ambits (10 km (squares), 20 km (triangles), and 60 km (diamonds)) from 1985 to 1994. Error bars (± SE) around values of PC(t) in Fig. 8*b* are from jackknifing of the data and reflect sampling uncertainty.



As expected, cod exhibited changes in patch structure and contact with neighbours during the period of changing spatial distribution and stock collapse in the 1990s. Our analysis indicates that changes in spatial structure occurred in three ways: (*i*) reduction in the number of patches from 1990, (*ii*) reduction in the size (spatial extent and number of individuals) of remaining patches from 1992, and (*iii*) reduction in local density and small-scale contact with neighbours from 1993. There is support for our interpretation from several sources. Lilly (1994) and Hutchings (1996) have both used density plots to show that patches of high cod density declined in the 1990s. The decline in the number of patches occurred from the north, resulting in a southward shift in the overall distribution of the cod, as indicated by density centroids (Rose et al. 1994) or ellipses (Atkinson et al. 1997). Warren (1997) showed that spatial structure measured from variograms had broken down (was not distinguishable from random) in the northern and central subareas by 1992 but

Fig. 9. Conceptual basin models (after MacCall 1990) of Atlantic cod distribution (see text for details). (*a*) Basin model with no latitudinal gradient in habitat suitability. The stock level indicated by the solid line is equivalent to the situation of the cod stock in the 1980s. Several patches with similar structure are present. The dashed line shows the predicted effect of a decrease in stock size with no change in habitat suitability. (*b*) Basin model showing hypothesised change in habitat suitability in the 1990s. Habitat suitability is decreased in the north and fish "flow down" to replenish the remaining patches. The solid line represents stock size and structure in 1992. Note that the size and shape of the main patch are similar to those in Fig. 9*a*, even though population size (analogous to fluid volume) is much lower. As stock size is further reduced (dashed line), patch extent and local density decrease.



was still evident in the south. Ours is the first formal description of a reduction in patch size based on fall survey data, but Rose and Kulka (1999) found that the area occupied by high densities of cod in an overwintering – spring migration area became reduced as stock size declined from 1990 to 1994. Rose and Kulka (1999) also showed, as we did, that local density (and hence, contact at small scales) was maintained, and even increased, as stock biomass declined.

Our interpretation of a three-step change in cod spatial structure during the period of stock decline fits within the framework of the conceptual basin model proposed by MacCall (1990). In this model, variations in habitat suitability are described by an irregular basin (Fig. 9). The fish population fills this basin like a liquid under the influence of gravity to a level determined by the stock size. As stock size decreases, the level in the basin falls, so fish only remain in patches corresponding to areas of high habitat suitability (depressions in the basin model). If the stock continues to decline, fish in less suitable habitats disappear first.

It is widely acknowledged that overfishing was the major cause of the decline in the Newfoundland cod population, but we do not believe that the observed changes in cod spatial structure can be explained by overfishing alone, as suggested by Hutchings (1996). Under this model, as stock size declined, individual patches would be reduced at a rate proportional to the fishing effort. This does not fit with the observation that patches disappeared first in the north, where fishing pressure was lower (Rose et al. 2000), but persisted further south even up to the final collapse. Instead, we believe that other factors, perhaps indexed by anomalously low water temperatures (Rose et al. 1994) and changing distributions of important prey species (Carscadden and Nakashima 1997), affected cod habitat suitability in the early 1990s. Changing habitat suitability would alter the topography of the basin model (Fig. 9). In keeping with this interpretation, the loss of patches from the north was probably due to a decrease in habitat suitability related to adverse environmental conditions. Migrants from the north could then have joined and temporarily replenished patches to the south (Rose et al. 2000), which is equivalent to flow down the habitat preference gradient in the present model (Fig. 9*b*). This model accounts for the observed maintenance of patch structure and local density even after the population had declined to very low levels in 1992.

Observations of changes in spatial distribution of cod as the stock declined may be used as a guide to possible changes as the stock recovers. We speculate that cod spatial distribution during recovery will reverse the changes that occurred during the decline. Local density will increase first, followed by an increase in patch size and extent, with the final step being an increase in the number of patches. Whether the cod will return to their historical distribution pattern is dependent on whether hypothesised changes in habitat suitability are also reversible.

Contact statistics can be used to measure spatial association, but further work is required to determine an appropriate null model for assessing significance. The four alternative null models that we presented represent different null hypotheses about the process generating spatial structure. Random shift and random clump null models assume that spatial structure in species A is generated independently of species B. Power to detect significance using these models was low due to constraints imposed by the size of the sampled area. At large scales, some random arrangements would always align the patches of the two types of individuals more closely than the pattern observed in the data. Null models that randomised individual increments of density or density values were not constrained by maintaining spatial structure, and the possible set of randomisations was much greater. However, because these null models change the second-order properties of one of the distributions, they are not tests of independence (Roxburgh and Chesson 1998). As long as this violation is stated explicitly, the use of such random models may still provide useful ecological information. For example, the random values null model that we used to calculate confidence intervals for our trawl data was based on the hypothesis that sample estimates of cod density were independent and that second-order spatial properties were generated only by aggregation of cod in response to capelin. This hypothesis is valid if schools of cod are smaller than the separation between trawl sample points and do not aggregate except in response to capelin. O'Driscoll (1998) randomised individual seabirds (random individuals null model) to test the hypothesis that more birds were associated with schools of fish than would be expected if the seabirds were distributed randomly. This hypothesis becomes biologically interpretable if seabirds are foraging individually and aggregating in response to fish schools.

Cod were only broadly associated with capelin prey in the bottom-trawl data from 1985 to 1994. This agrees with the interpretation of Lilly (1994) based on visual comparison of plots of cod and capelin distribution from 1985 to 1992. Scale of maximum association between cod and capelin decreased in 1989 and then remained relatively constant during the 1990s, reflecting common changes in the distribution patterns of the two species. Although distributions of both cod (Atkinson et al. 1997) and capelin (Carscadden and Nakashima 1997) contracted in the 1990s, the area occupied by the remaining fish was similar (Lilly 1994). Offshore concentrations of capelin shifted southward (Carscadden and Nakashima 1997) over the same time period that cod became concentrated in the southern part of their former range (Rose et al. 2000). The potential contact between cod and capelin within hypothetical cod daily ambits of 10, 20, and 60 km did not decrease in the 1990s, suggesting that changes in the distribution of both cod and capelin did not negatively affect the likelihood of cod encountering capelin prey. This conclusion is supported by analysis of cod stomach contents. Lilly (1994) reported that feeding on capelin did not seem to decline in the early 1990s, except in the north of our survey area. The southward shift in cod distribution observed in the 1990s may have been driven in part by a need for cod to maintain broad-scale association with capelin.

There was no positive association between cod and capelin at small scales (10–100 km). There are several possible explanations for this lack of association. First, trawl surveys were conducted in the late fall, which is not regarded as the period of peak feeding on capelin by cod (Turuk 1968), although capelin were commonly found in stomachs of cod caught in trawls at this time (Lilly 1987, 1994). Second, the Engels bottom-trawl may not have adequately sampled capelin distribution. Capelin are a pelagic species and will only be caught in a bottom-trawl when concentrated near the bottom. There is also evidence that the Engels trawl is not an efficient gear for catching capelin. Estimates of trawlable biomass of capelin from fall surveys increased by an order of magnitude following a change of gear type from an Engels trawl to a Campelen trawl in 1995 (Lilly 1998). Third, Horne and Schneider (1994) have suggested, based on a physiological model, that cod do not have to be coupled spatially with capelin at small scales but can function as "sit and wait" predators. Even though cod were not closely associated with capelin, distributions were broadly similar and there was potential for contact with prey at scales similar to the scale of the hypothetical daily cod ambit.

Estimates of contact based on bottom-trawl data were relative because of uncertainties about the ability of the gear to adequately sample fish, particularly capelin. Concerns have also been expressed (e.g., Warren 1997) that changes in the timing of the trawl survey between years and also the time taken to complete the survey may influence observed spatial structure. Despite these limitations, our analysis provides a formal statistical assessment of changes in cod distribution and association with capelin over the period of the cod stock collapse. Our interpretation adds to the knowledge of the way that the Newfoundland cod declined and provides a predictive framework for the changes that we might expect to see as the stock recovers. With improved sampling methods, such as acoustic techniques that allow measurements of absolute fish densities at small scales, it may be possible to estimate both potential and realised contact at yet finer scales and apply these values to predictive dynamic models of social behaviour or foraging.

Acknowledgements

We thank Alec MacCall, Philip Dixon, Gareth Lawson, Gordie Swartzman, and three anonymous reviewers for comments on this manuscript. This work was funded by a New Zealand Foundation for Research Science and Technology postdoctoral Fellowship awarded to the first author and the Natural Sciences and Engineering Research Council of Canada Chair in Fisheries Conservation.

References

Andersen, M. 1992. Spatial analysis of two-species interactions. Oecologia, **91**: 134–140.

Andrew, N.L., and Mapstone, B.D. 1987. Sampling and the description of spatial pattern in marine ecology. Oceanogr. Mar. Biol. Annu. Rev. **25**: 39–90.

Atkinson, D.B., Rose, G.A., Murphy, E.F., and Bishop, C.A. 1997. Distribution changes and abundance of northern cod (*Gadus morhua*), 1981–1993. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 132–138.

Bult, T.P., Haedrich, R.L., and Schneider, D.C. 1998. New technique describing spatial scaling and habitat selection in riverine habitats. Regul. Rivers Res. Manage. **14**: 107–118.

Carscadden, J., and Nakashima, B.S. 1997. Abundance and changes in distribution, biology, and behavior of capelin in response to cooler waters of the 1990s. *In* Forage fishes in marine ecosystems. Lowell Wakefield Symposium Series. University of Alaska Sea Grant College Program, Fairbanks, Alaska. pp. 457–468.

Coomes, D.A., Rees, M., and Turnbull, L. 1999. Identifying aggregation and association in fully mapped spatial data. Ecology, **80**: 554–565.

Cressie, N.A.C. 1991. Statistics for spatial data. John Wiley & Sons, New York.

Haase, P. 1995. Spatial pattern analysis in ecology based on Ripley's *K*-function: introduction and methods of edge correction. J. Veg. Sci. **6**: 575–582.

Horne, J.K., and Schneider, D.C. 1994. Lack of spatial coherence of predators with prey: a bioenergetic explanation for Atlantic cod feeding on capelin. J. Fish Biol. **45**(Suppl. A): 191–207.

Hutchings, J.A. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**: 943–962.

Legendre, P., and Fortin, M.J. 1989. Spatial pattern and ecological analysis. Vegetatio, **80**: 107–138.

Levin, S.A. 1992. The problem of pattern and scale in ecology. Ecology, **73**: 1943–1967.

Lilly, G.R. 1987. Interactions between Atlantic cod (*Gadus morhua*) and capelin (*Mallotus villosus*) off Labrador and eastern Newfoundland: a review. Can. Tech. Rep. Fish. Aquat. Sci. No. 1567.

Lilly, G.R. 1994. Predation by Atlantic cod on capelin on the southern Labrador and Northeast Newfoundland shelves during a period of changing spatial distributions. ICES Mar. Sci. Symp. **198**: 600–611.

Lilly, G.R. 1998. By-catches of capelin during spring and autumn bottom-trawl surveys in divisions 2J3KL in 1997. *In* Capelin in SA2 + div. 3KL. Can. Stock Assess. Secretariat Res. Doc. No. 98/63. pp. 79–113.

Lloyd, M. 1967. Mean crowding. J. Anim. Ecol. **36**: 1–30.

Logerwell, E.A., Hewitt, R.P., and Demer, D.A. 1998. Scale-dependent spatial variance patterns and correlations of seabirds and prey in the southeastern Bering Sea as revealed by spectral analysis. Ecography, **21**: 212–223.

Lotwick, H.W., and Silverman, B.W. 1982. Methods for analysing spatial processes of several types of points. J. R. Stat. Soc. Ser. B (Methodol.), **44**: 406–413.

MacCall, A.D. 1990. Dynamic geography of marine populations. University of Washington Press, Seattle, Wash.

Malatesta, R.J., Auster, P.J., and Carlin, B.P. 1992. Analysis of transect data for microhabitat correlations and faunal patchiness. Mar. Ecol. Prog. Ser. **87**: 189–195.

Manly, B.F.J. 1997. Randomization, bootstrap and Monte Carlo methods in biology. 2nd ed. Chapman and Hall, London, U.K.

Muotka, T., and Penttinen, A. 1994. Detecting small-scale spatial patterns in lotic predator–prey relationships: statistical methods and a case study. Can. J. Fish. Aquat. Sci. **51**: 2210–2218.

O'Driscoll, R.L. 1998. Description of spatial pattern in seabird distributions along line transects using neighbour *K* statistics. Mar. Ecol. Prog. Ser. **165**: 81–94.

Platt, T., and Denman, K.L. 1975. Spectral analysis in ecology. Annu. Rev. Ecol. Syst. **6**: 189–210.

Ripley, B.D. 1981. Spatial statistics. John Wiley & Sons, New York.

Rose, G.A., and Kulka, D.W. 1999. Hyperaggregation of fish and fisheries: how catch-per-unit-effort increased as the northern cod (*Gadus morhua*) declined. Can. J. Fish. Aquat. Sci. **56**(Suppl. 1): 118–127.

Rose, G.A., Atkinson, B.A., Baird, J., Bishop, C.A., and Kulka, D.W. 1994. Changes in distribution of Atlantic cod and thermal variations in Newfoundland waters, 1980–1992. ICES Mar. Sci. Symp. **198**: 542–552.

Rose, G.A., deYoung, B., and Colbourne, E.B. 1995. Cod (*Gadus morhua* L.) migration speeds and transport relative to currents on the north-east Newfoundland Shelf. ICES J. Mar. Sci. **52**: 903–913.

Rose, G.A., deYoung, B., Kulka, D.W., Goddard, S.V., and Fletcher, G.L. 2000. Distribution shifts and overfishing the northern cod (*Gadus morhus*): a view from the ocean. Can. J. Fish. Aquat. Sci. **57**: 644–663.

Rossi, R.E., Mulla, D.J., Journel, A.G., and Franz, E.H. 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. Ecol. Monogr. **62**: 277–314.

Roxburgh, S.H., and Chesson, P. 1998. A new method for detecting species associations with spatially autocorrelated data. Ecology, **79**: 2180–2192.

Schneider, D.C. 1994. Quantitative ecology: spatial and temporal scaling. Academic Press, San Diego, Calif.

Schneider, D.C., and Piatt, J.F. 1986. Scale-dependent correlation of seabirds with schooling fish in a coastal ecosystem. Mar. Ecol. Prog. Ser. **32**: 237–246.

Schneider, D.C., Gagnon, J.-M., and Gilkinson, K.D. 1987. Patchiness of epibenthic megafauna on the outer Grand Banks of Newfoundland. Mar. Ecol. Prog. Ser. **39**: 1–13.

Turuk, T.N. 1968. Seasonal changes of cod feeding in the Labrador and Newfoundland area in 1964–1966. Tr. PINRO, **23**: 370–382.

Warren, W.G. 1997. Changes in the within-survey spatio-temporal structure of the northern cod (*Gadus morhua*) population, 1985–1992. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 139–148.

Effects of fish predation in erosional and depositional habitats in a temperate stream

Jordan Rosenfeld

Abstract: Combined effects of predation by benthic and drift-foraging fish (prickly sculpin (*Cottus asper*) and coho salmon (*Onchorhynchus kisutch*) parr) on benthic invertebrate community and trophic structure were evaluated in Mayfly Creek, a previously fishless stream in the Coast Range Mountains of British Columbia. The role of microhabitat (substrate) in mediating predation effects was assessed by comparing invertebrate community structure on unglazed ceramic tiles and gravel baskets nested within enclosures. The role of macrohabitat was evaluated by placing enclosures in pool and riffle habitats. Effects of fish predation were most pronounced on tile substrate and in riffle habitat and least pronounced on gravel substrate in pool habitat. The presence of fish caused a decrease in abundance of larger-bodied herbivores (primarily the mayflies *Ameletus* and *Baetis*) and had positive indirect effects on algae and smaller invertebrates (primarily Orthocladiinae chironomids and nemourid stoneflies), probably through competitive release. In contrast with herbivores, detritivorous invertebrates were less influenced by fish predation and more highly correlated with the abundance of organic detritus. The distribution and abundance of detritivores in Mayfly Creek appear to be primarily influenced by bottom-up forces (implying resource limitation), while grazers in algal-based food chains are more strongly influenced by top-down effects (fish predation).

Résumé : Les effets combinés de la prédation exercée par les poissons qui se nourrissent sur le fond et dans la colonne d'eau (chabot piquant (*Cottus asper*) et tacon de coho (*Oncorhynchus kisutch*)) sur la communauté d'invertébrés benthiques et la structure trophique ont été évalués dans le crique Mayfly, cours d'eau autrefois dépourvu de poissons de la chaîne côtière de la Colombie-Britannique. Le rôle du microhabitat (substrat) comme agent de médiation des effets de la prédation a été évalué en comparant la structure de la communauté d'invertébrés sur des tuiles de céramique non vernissées et dans des paniers de gravier installés dans des enclos. Le rôle du macrohabitat a été évalué en plaçant les enclos dans des fosses et des radiers. Les effets de la prédation exercée par les poissons étaient plus marqués sur les tuiles et dans les radiers et moins marqués sur le gravier dans les fosses. La présence de poissons a entraîné une diminution de l'abondance des herbivores de grande taille (surtout les éphémères des genres *Ameletus* et *Baetis*), et avait des effets positifs indirects sur les algues et les invertébrés de petite taille (principalement des chironomides de la famille des Orthocladiinae et des plécoptères de la famille des Nemuridae), effets probablement attribuables à une libération compétitive. Par contraste avec les herbivores, la prédation exercée par les poissons influait moins sur les invertébrés détritivores, qui présentaient une corrélation plus marquée avec l'abondance des détritus organiques. Il semble que la distribution et l'abondance des détritivores dans le crique Mayfly soient influencées principalement par des forces ascendantes (ce qui implique une limitation des ressources), tandis que les effets descendants (prédation exercée par le poisson) influent plus fortement sur les brouteurs dans les chaînes trophiques basées sur les algues.

[Traduit par la Rédaction]

Introduction

Predation can be a powerful force shaping the structure of prey communities (Hunter and Price 1992). Although several recent studies have demonstrated strong effects of fish predation on invertebrate community structure in streams (e.g., Power 1990; Bechara et al. 1992; Flecker 1992), many others have found at best weak effects (Allan 1982; Flecker and Allan 1984; Culp 1986), and much of the variance in predator impacts in streams remains unexplained (Wooster 1994). The degree to which predation influences community structure will depend on a variety of biotic and abiotic factors (Hunter and Price 1992), including the attributes (e.g., size, intrinsic rate of growth) of the primary producer trophic level (Power 1992*a*), the functional role and spatial distribution of dominant predator taxa (Dahl and Greenberg 1996), and differences in prey movement rates (Cooper et al. 1990; Englund 1997) between habitat patches.In physically complex systems like streams, substrate heterogeneity can also play a key role in mediating predation effects through the availability of refuges. For example, fish predation caused a strong trophic cascade on smooth boulder substrate but not over gravel in the Eel River, California, which was attributed to the availability of interstitial refuges in gravel substrate (Power 1992*b*). Independent of substrate heterogeneity, the differentiation of aquatic invertebrates into discrete functional feeding groups may also affect their vulnerability to shared predators depending on whether the resources that they consume are in habitats that are more or less exposed to

Received September 2, 1999. Accepted March 29, 2000.
J15342

J. Rosenfeld. Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

[1] Present address: B.C. Ministry of Fisheries, Fisheries Research, 2204 Main Mall, Vancouver, BC V6T 1Z4, Canada. e-mail: jordan.rosenfeld@gems4.gov.bc.ca

fish predation (Kohler and McPeek 1989; Bechara et al. 1992). In particular, herbivorous invertebrates may be constrained to foraging on algae on the upper surfaces of rocks, while detritivores feed on detrital accumulations in substrate interstices, leading to the prediction that direct and indirect fish predation effects should be greatest in habitats with an algal carbon base, i.e., where the majority of food resources are on upper substrate surfaces.

Habitat and substrate heterogeneity, in addition to influencing the availability of refuges, will also affect the relative abundances of algal and detrital organic matter in different habitat types. (These two sources of organic carbon for invertebrates in streams (detrital organic matter versus living plant tissue) will, for simplicity, subsequently be referred to as algal and detrital carbon.) Because of the nature of bedload transport in streams, depositional habitats (pools) and microhabitats (substrate interstices) tend to accumulate detrital carbon (Rabeni and Minshall 1977; Huryn and Wallace 1987), while erosional habitats (riffles) and microhabitats (upper rock surfaces) tend to support algal production (Rosenfeld and Hudson 1997). If functional groups of invertebrates differ in their vulnerability to predation, then small-scale differences in the carbon base of patches within a stream may lead to differences in the impact of local predation in a spatially heterogeneous environment.

Most studies of fish effects in streams have been within fish-bearing streams where fish effects are likely to be smaller or expressed as differences in behavioural responses to predation risk (e.g., McIntosh and Townsend 1996) rather than as radical changes in species composition. An alternative approach for studying the effects of fish predation is to examine the impact of introducing a foreign predator to a previously fishless system. For example, the first study to clearly demonstrate the impact of fish predation on zooplankton was based on the introduction of freshwater herring to a lake without fish adapted to planktivory (Brooks and Dodson 1965). While the impact of fish predation on a naive invertebrate community may be exaggerated (Neill 1994), effects may be clearer than in a fish-bearing system, and the mechanisms and pathways of predator impacts on prey should be similar (Rosenfeld 2000).

The experiment described in this study examined the impact of fish predation on invertebrate community structure in a previously fishless stream. Specific objectives were to assess (*i*) whether membership in different invertebrate functional feeding groups influenced vulnerability to predation and (*ii*) how habitat (pool versus riffle) and microhabitat (gravel versus tile) modified local predator impacts. Simplistic expectations for a three-link food chain (algae/detritus–invertebrates–fish) were that predator impacts should be greatest on simple substrates lacking refugia and on grazing invertebrates in predominantly algal-based food chains.

Materials and methods

Study site

The study was carried out in Mayfly Creek, a second-order stream in the Coastal Western Hemlock biogeoclimatic zone of British Columbia. Mayfly Creek is located 60 km northeast of the city of Vancouver in the University of British Columbia Malcolm Knapp Research Forest (49°18′40″, 122°32′40″) at an elevation of 350 m in the Coast Range Mountains. Streams in coastal British Columbia receive large amounts of rainfall and tend to be moderately oligotrophic. Mayfly Creek flows through second-growth forest with a partially open canopy dominated by western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and Douglas-fir (*Pseudotsuga menziesii*), with red alder (*Alnus rubra*) and salmonberry (*Rubus spectabilis*) common in the riparian zone. Experiments were performed in the upper fishless reach of Mayfly Creek above a series of cascades, which provide a barrier to resident cutthroat trout (*Oncorhynchus clarki*). Research was done during summer baseflow when minimum discharge can be as low as 30 $L \cdot s^{-1}$ and wetted width is between 2 and 5 m. The stream gradient in this reach averages 1%, and the substratum is dominated by gravel, cobble, and sand in a channel with well-developed pools and riffles. Canopy cover is moderate with occasional gaps, and the stream is cool, rarely exceeding 17.5°C (Richardson 1991).

Experimental design

Experimental units consisted of five enclosures placed in separate channel units (three in riffles, two in pools) spaced 20–100 m apart over a 400-m reach of stream. Enclosures were constructed of a wooden frame supported by 19-mm-diameter, concrete-reinforcing rods (iron rebar), with plywood sides embedded in the stream channel and 6.5-mm galvanized steel mesh across the upstream and downstream ends (Fig. 1). The 6.5-mm-mesh opening was large enough to permit free movement of most stream organisms, with the exception of larger larvae of the tailed frog (*Ascaphus truei*), but small enough to retain fish. Enclosures were 6 m long, 2 m wide, and 1 m high and were divided internally into a larger upstream section and two lower sections (Fig. 1). Each lower section was 2 m long and 1 m wide. The upper sections of all enclosures were in pools and the lower sections were either in slow riffles, where current velocity averaged 16.4 ± 1.8 $cm \cdot s^{-1}$ (range 11–22 $cm \cdot s^{-1}$), sufficient to prevent deposition of fine particulate organic matter on upper substratum surfaces, or in pools with much lower current velocity (4.9 ± 1.2 $cm \cdot s^{-1}$, range 2–7 $cm \cdot s^{-1}$). Velocities were measured once during baseflow at four points along transects across lower enclosure sections using a Marsh-McBirney model 2000 flowmeter.

Lower enclosure sections were used for fish treatments and controls. Design and interpretation of enclosure experiments in streams are complicated by naturally high background movement rates of prey that may swamp the effects of predation in inappropriately small enclosures (Cooper et al. 1990; Englund 1997), leading to underestimates of predation effects as an artifact of enclosure size. This was of concern in fishless Mayfly Creek, where there was an unknown potential for invertebrate densities and drift rates to be higher than in a fish-bearing stream. To minimize the probability of fish effects in lower enclosure sections being obscured by high immigration rates of prey from fishless areas upstream, upper enclosure sections were also stocked with fish.

To evaluate fish predation effects in different microhabitat types, four 15 × 15 cm unglazed ceramic tiles and five open gravel baskets were placed in each lower enclosure section for colonization by algae and invertebrates. Tiles were used in this experiment as rock surrogates, since regularity in size, shape, and surface texture reduces variance, and benthic community structure on unglazed ceramic tiles has been shown to be similar to that on rock substrate (Lamberti and Resh 1985). Gravel baskets were 30 cm long, 20 cm wide, 5 cm deep, and were made of 6.5-mm-mesh hardware cloth buried flush with the streambed in each lower enclosure section. Baskets were filled with approximately 2.5 L of 4- to 7-cm-diameter river-washed gravel; an additional 1 L of sand and fine gravel was added to partially fill gravel interstices.

One lower section of each enclosure was stocked with coho salmon (*Onchorhynchus kisutch*) parr (mean (± SD) length 50.2 ± 6.5 mm, mean weight 1.6 ± 0.6 g) and prickly sculpin (*Cottus*

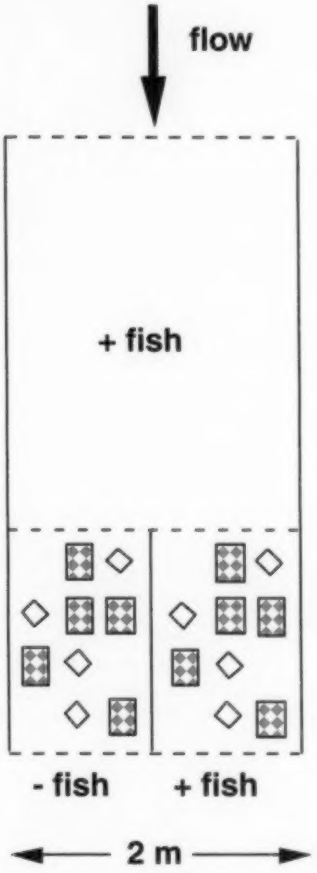


Fig. 1. Design of experimental enclosures. Stippled rectangles represent gravel baskets, diamonds represent tiles, and broken lines denote permeable mesh.

asper) (mean length 72.8 ± 9.8 mm, mean weight 4.3 ± 1.9 g) at a density of 2 and 1 fish·m^{-2}, respectively, and the other lower section was left as a fishless control. Each upper enclosure section was stocked with fish at the same density; coho in upper sections were partially restocked when the smallest fish escaped through the 6.5-mm mesh, so that final densities of coho in upper sections averaged 3 fish·m^{-2}. Average coho and sculpin weight in all enclosure sections combined increased by 95 and 13%, respectively, during the course of the experiment, with a range in final weight of 1.2–9.8 g for coho and 2.6–8.9 g for sculpin. Positive growth suggests that fish densities were not excessive for the productivity of the system. The appropriate ambient density of fish was determined by electrofishing reaches in nearby streams of similar size and water chemistry (J. Rosenfeld, unpublished data). Coho and sculpin used during the experiment were collected from the Allouette River downstream of Mayfly Creek by electrofishing or baited minnow traps.

Fish were stocked during May 18–20, 1993, 1 week after substrates were placed in enclosures. Lengths and weights of all fish were measured at the beginning and end of the experiment. To ensure that upper Mayfly Creek remained fishless, sculpin were sexed using the morphology of the anal papilla, and only males were used. Several impassable falls downstream ensured that any escaped coho would be unable to return to the stream to spawn after running to the ocean. Juvenile coho were used rather than resident cutthroat trout to ensure that escaped fish would not colonize the stream. Fish were collected from enclosures by electroshocking at the end of the experiment, following removal of artificial substrates. Voltage was turned up to 700 V and the entire enclosure thoroughly shocked to ensure mortality of any fish that escaped capture.

Tiles and gravel baskets were sampled from August 26 to 31, 101 days after fish introduction, by carefully lifting each substrate off of the stream bottom into a 150-μm-mesh Surber sampler held immediately downstream. Invertebrates were rinsed off of individual tiles into a bucket using a wash bottle and brush, and the contents of the Surber sampler were also backwashed into the same bucket. After collection with a Surber sampler, contents of individual gravel baskets were emptied into a 10-L basin and agitated, and water in the basin was repeatedly decanted into a 150-μm net until the gravel was clean of organic matter and invertebrates. Water containing invertebrates and detritus from gravel and tile baskets was separately filtered through 150-μm-mesh sieves and the filter contents were preserved in 5% formalin. Invertebrates larger than 1 mm were later sorted from samples in the laboratory and identified to genus using Merritt and Cummins (1984), with the exception of chironomids, which were identified to subfamily. Samples from gravel baskets were split into quarters when the volume of detritus in the sample exceeded 200 mL. Chironomids were sorted and counted from randomly chosen subsamples until a minimum of 200 chironomids were counted. Counts were only split for chironomids, which were the most abundant invertebrate; all other taxa were completely sorted from each sample. Lengths of a total 26 000 invertebrates were measured to the nearest 0.05 mm using a digitizing system and a Wild M5 dissecting scope equipped with a drawing tube.

Chlorophyll *a* was measured only on gravel substrata. Five pieces of gravel were randomly removed from the surface of separate gravel baskets on each enclosure side, and algae on individual gravel pieces were extracted for chlorophyll *a* in 90% acetone in 250-mL glass jars on ice in the dark overnight. Chlorophyll *a* fluorescence was measured the next day using a Turner Designs model 10-005 R fluorometer. Chlorophyll *a* abundance was corrected for the upper surface area of each stone (measured by tracing outlines on paper) and is expressed as milligrams per square metre. Chlorophyll *a* was not measured on tiles because of logistic difficulties subsampling tiles for chlorophyll *a* and because periphyton abundance was visually similar on both tile and gravel. Chlorophyll *a* measurements from a subsequent predation experiment in the same enclosures the following year demonstrated that chlorophyll *a* levels were roughly equivalent on both substrate types (2.9 ± 1.0 mg·m^{-2} on tile, 4.5 ± 1.7 mg·m^{-2} on gravel; Rosenfeld 1997*a*).

Ash-free dry mass of all organic matter greater than 150 μm associated with each substrate was measured by drying organic matter from each sample to a constant weight in a preashed aluminium boat at 75°C and then combusting samples in a muffle furnace for 4 h at 550°C. Organic matter on tiles in riffles consisted primarily of periphyton and fine particulate organic matter, while organic matter in gravel baskets and on tiles in pools also included coarser detritus.

Data analysis

Data analysis was performed using PC SAS version 6.03. Invertebrate abundance data were (log + 1) transformed, proportional data were arsine square root transformed, and predator impact (PI) indices (described below) were square root transformed to maximize normality of distributions and equalize variances. Statistical significance was accepted at $\alpha = 0.05$. Fish effects on chlorophyll *a* were analyzed as a paired t test (the difference between predator and control treatments within each enclosure) for pools ($n = 2$) and riffles ($n = 3$) separately. Fish effects on total invertebrate abundance and individual taxa were analyzed as a split-plot design with enclosures as blocks ($n = 5$), fish presence/absence as the main effect (two levels within each block), and substrate (two levels) as a subplot within each main plot (total $n = 20$) (Snedecor and Cochran 1989); habitat type (pool versus riffle) was not included in this analysis because of insufficient degrees of freedom. Critical significance levels for evaluating the significance of fish effects on individual taxa were adjusted with a sequential Bonferroni correction (Sokal and Rohlf 1997) for multiple tests (first critical $\alpha = 0.05/9 = 0.0056$). If there was a significant fish by substrate interaction, the effect of fish predation was analyzed separately for tile and gravel substrate as a randomized block design without replication (Sokal and Rohlf 1997) ($n = 5$ blocks, two fish levels, total $n = 10$). Invertebrate – organic matter relationships were evaluated using analysis of covariance (ANCOVA) with organic matter as the covariate. In order to simplify interpretation of the results, untransformed data were used for ANCOVA of total invertebrate – organic matter relationships (total $n = 20$), since distribution of residuals from regression with untransformed data was not significantly different from normal (Shapiro–Wilke normality test, $W = 0.93$, $P = 0.40$).

Because differences in densities between taxa may lead to biases in objectively scaling predation effects, a modification of the PI index described by Cooper et al. (1990) was used to assess predation effects on total invertebrate abundance. In addition to standardizing for differences in density between taxa, the PI index has the advantage of equivalently scaling increases and decreases relative to a control. PI is calculated as the natural logarithm of the ratio of prey densities in predator treatments to prey densities in controls ($\mathrm{PI} = \ln(N_\mathrm{P}/N_\mathrm{C})$. The negative sign used by Cooper et al. (1990) was dropped from the PI expression so that a positive or negative PI corresponds to an increase or decrease in prey abundance in the presence of predators.

Differences in community structure between combined substrate and habitat types were evaluated using principal components analysis. The correlation matrix was used as this reduces the influence of invertebrate abundance and has been demonstrated to give the most consistent results in ordination. In order to provide sufficient data points for a meaningful analysis of community structure, data from individual tiles and gravel baskets were used for the ordination rather than the enclosure means.

The contribution of fish predation as a source of variation in total invertebrate abundance and abundance of individual taxa was calculated using variance components, as described by Sokal and Rohlf (1997), for tile and gravel separately (randomized block design without replication, $n = 5$ blocks, two fish levels, total $n = 10$). Abundance of invertebrates and organic carbon covaried at two distinct spatial scales in the experimental design: within enclosures (between replicate substrates) and between enclosures. The correlation of invertebrate abundance with organic matter, for all invertebrates and taxa individually, was partitioned into within- and between-enclosure components for gravel and tile separately using sequential sums of squares. The variance in abundance due to organic matter within enclosures was calculated as the residual variance after enclosure effects (the block) were used to remove the effects of average organic matter abundance per enclosure in multiple regression ($n = 40$ for tile, four tiles per enclosure side; $n = 50$ for gravel, five gravel baskets per enclosure side). The variance in invertebrate abundance due to differences in average organic matter per enclosure was calculated by subtracting the variance due to organic matter within enclosures from the total variance due to organic matter both within and between enclosures. The regression mean square was used as the error term to evaluate the significance of organic matter both within and between enclosures.

Research in aquatic systems has demonstrated that large-bodied invertebrate taxa are typically more vulnerable to fish predation (Allan 1978). To test for differences in predation effects as a function of prey size, invertebrates were divided into small (<3 mm), medium (≥3 mm, <6 mm), and large (≥6 mm) size-classes based on total length. These size-class divisions roughly correspond to <0.1, 0.1–1.0, and >1.0 mg weight-classes based on length–weight regressions from Smock (1980). These weight-classes were identified by Allan (1981) as being approximately representative of the vulnerability of invertebrates to drift-foraging fish; invertebrates <0.1 mg show a lower vulnerability to fish predation and propensity for diurnal drift periodicity, whereas those >1 mg are preferentially selected by fish and tend to drift primarily at night (Allan 1984). Because abundances of different size-classes of invertebrates from an individual substrate are not independent, the effect of fish predation on invertebrate abundance in different size-classes was analyzed as a split-plot design with enclosures as blocks ($n = 5$), fish presence (two levels) as the main effect, and size (three levels) as a subplot (or repeated measure) within a plot (total $n = 30$) for tile and gravel separately (Snedecor and Cochran 1989). A split-plot design is an appropriate method for dealing with the nonindependence of repeated measures when the order of replicate measurements is unimportant (Snedecor and Cochrane 1989).

Results

Fish effects on chlorophyll *a*

Impacts of fish on algae were consistent with the expectation of greater fish effects in habitats dominated by herbivorous invertebrates. Chlorophyll *a* was significantly higher on gravel substrate in riffles in the presence of fish (paired t test, $t_2 = 4.41$, $P = 0.05$) (Fig. 2), but there was no apparent effect of fish on chlorophyll *a* in pools (paired t test, $t_1 = 0.66$, $P = 0.63$). Variance of chlorophyll *a* within enclosures was also significantly lower in the absence of fish in riffles (paired t test, $t_2 = 6.1$, $P = 0.03$) (Fig. 2), indicating more uniform grazing activity by herbivores in the absence of predation, but variance was unaffected in pools (paired t test, $t_1 = -0.96$, $P = 0.52$).

Invertebrate community structure and organic matter relationships

The combined ordination of substrate samples ($n = 50$ for gravel, $n = 40$ for tile) demonstrated that invertebrate communities on different substrate and habitat types are well separated (Fig. 3). The first principal component (PC1) explained 46.7% of the variation in the data set, and the second principal component (PC2) explained an additional 19.6%. There is a positive correlation between PC1 and the abundance of primarily detritivore taxa (e.g., *Despaxia* sp., *Zapada* sp., *Paraleptophlebia* sp., and Tipulidae) (see axis labels in Fig. 3); PC1 appears to be an axis representing detritivore abundance and separates tile from gravel substrate, indicating that detritivores are proportionally more abundant on gravel substrate. PC2 is positively correlated with the abundance of grazing mayflies and negatively correlated with the abundance of chironomids and appears to be an axis separating pool and riffle habitats, indicating that grazing mayflies are proportionately more abundant in riffles.

A significant positive relationship was observed between total invertebrate abundance on tiles and organic matter (ANCOVA, $F_{1,7} = 30.1$, $P = 0.001$, total $n = 10$ fish for treatment means) (Fig. 4A), suggesting limitation of invertebrate abundance by organic carbon (periphyton and fine particulate detritus) on tile surfaces. There was also a significant difference in intercept for pools and riffles (ANCOVA,

Fig. 2. Chlorophyll *a* concentration (±1 SD) on gravel substrate in individual pool and riffle enclosures in the presence and absence of fish. Solid circles represent fish treatments and open circles represent fishless controls.

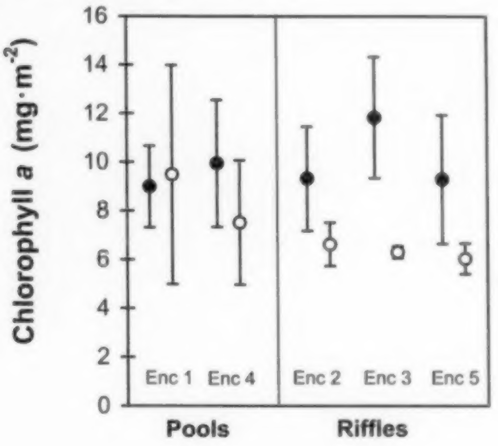


Fig. 3. Ordination of the four substrate–habitat combinations in species space (using only those taxa contributing to at least 1% of the invertebrate community); $n = 50$ for gravel and $n = 40$ for tile. Those taxa with the highest correlations of density with principal component score (correlation coefficient in parentheses) are labeled on the axes. Circles represent tiles in riffles, diamonds represent tiles in pools, triangles represent gravel in pools, and squares represent gravel in riffles.

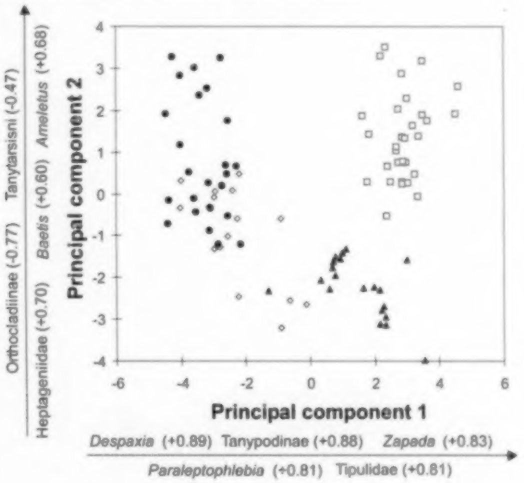
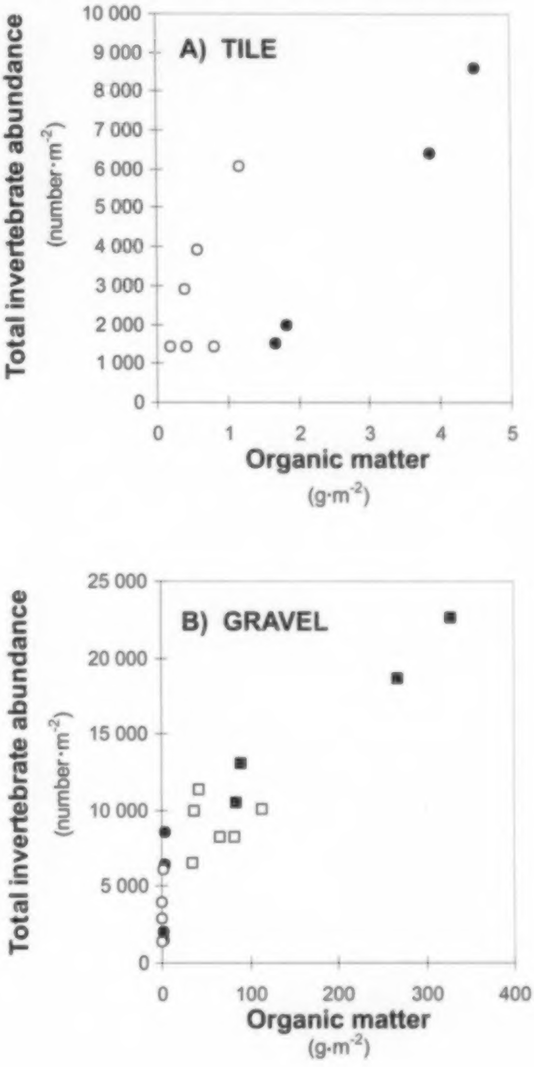


Fig. 4. Relationship between total invertebrate abundance and organic matter on (A) tiles and (B) gravel in pool and riffle habitat. Points represent enclosure treatment means ($n = 10$). The regression equation for tile is $N = 2520(\text{organic}) - 4240$ $(\text{habitat}) + 1400$, where habitat = 0 for riffle and 1 for pool. The regression equation for total invertebrate abundance on gravel is $N = 46.4(\text{organic}) + 6600$. Open circles represent tiles in riffles, solid circles represent tiles in pools, open squares represent gravel in riffles, and solid squares represent gravel in pools.



$F_{1,7} = 10.0$, $P = 0.016$), indicating that riffles supported more invertebrates for a given quantity of organic matter. There was a significant positive relationship between invertebrate abundance and organic matter on gravel substrate as well, but the slope of the relationship was significantly less than for tile (*t* test with unequal variances, Welch's approximate $t_9 = 17.04$, $P < 0.01$), and there was also no significant habitat (pool–riffle) effect for gravel (Fig. 4B), indicating substantial differences in invertebrate abundance – organic matter relationships between tile and gravel substrate.

The fish treatment had no detectable effect on the abundance of organic matter on gravel substrate but caused a small but significant increase in organic matter biomass (periphyton and fine detritus) on tiles (analysis of variance (ANOVA), $F_{1,5} = 11$, $P = 0.03$, total $n = 10$ for treatment means); mean organic matter on tiles (as ash-free dry

Table 1. Results of split-plot ANOVA for the effect of fish predation and substrate type on total invertebrate abundance (log transformed).

Effect	Source	df	SS	MS	*F*	*P*
Main	Enclosure	4	0.519	0.130	13.19	0.014*
	Fish	1	0.177	0.177	17.98	0.013*
	Main plot error[a]	4	0.039	0.010	0.58	0.685
Subplot	Substrate	1	1.822	1.822	107.59	0.0001**
	Fish × substrate	1	0.124	0.124	7.34	0.027*
	Subplot error[b]	8	0.136	0.017		

Note: *Significant at $\alpha = 0.05$; **significant at $\alpha = 0.01$.

[a] Main plot error = (enclosure × fish).

[b] Subplot error = enclosure × substrate + enclosure × fish × substrate).

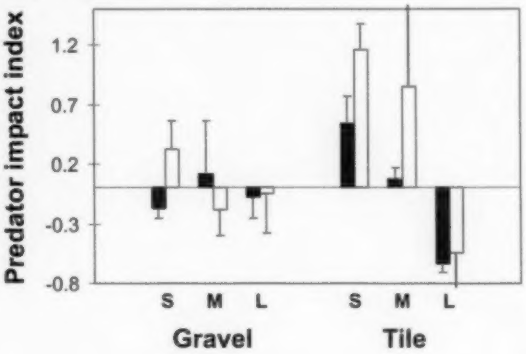


Fig. 5. PI index (±1 SD) for total invertebrate abundance in three size-classes, small (<3 mm), medium (≥3 mm, <6 mm), and large (≥6 mm), on tile and gravel substrate in pool ($n = 2$) and riffle ($n = 3$) habitat. Open bars represent riffles and solid bars represent pools.

weight) averaged 1.7 $g{\cdot}m^{-2}$ in the fish treatment and 1.4 $g{\cdot}m^{-2}$ in the control.

Mean organic matter biomass (primarily detrital carbon) was two orders of magnitude higher on gravel than on tiles (ANOVA, $F_{1,7} = 142.5$, $P = 0.0001$, total $n = 10$ for enclosure side means) and three to five times higher in pools than in riffles (ANOVA, $F_{1,7} = 11.9$, $P = 0.011$, $n = 10$), averaging 0.58 ± 0.36 $g{\cdot}m^{-2}$ on tiles in riffles, 2.98 ± 1.76 $g{\cdot}m^{-2}$ on tiles in pools, 62.8 ± 20.7 $g{\cdot}m^{-2}$ on gravel in riffles, and 193 ± 149 $g{\cdot}m^{-2}$ on gravel in pools.

Predation effects on total invertebrate abundance by size-class

Total abundance of invertebrates was significantly affected by the fish treatment (split-plot ANOVA) (Table 1), with a significant interaction between fish treatment and substrate type (Fig. 5; Table 1). As expected, different size-classes of invertebrates responded differently to the presence of fish, but the effects were substrate specific (Table 2). There was a significant fish by invertebrate size interaction on tile but no significant fish effects or interaction on gravel (split-plot ANOVA) (Table 2). Enclosures are treated as blocks, and the significant enclosure effect indicates differences in invertebrate abundance between enclosures; the highly significant size effect (Table 2) simply indicates that abundance of invertebrates differs greatly among size-classes, i.e., invertebrates were more abundant in the smaller size-classes.

Fish predation on tiles in riffles increased the abundance of the smaller and intermediate invertebrate size-classes (Fig. 5). On tiles in pools, the smallest size-class was also more abundant in the presence of fish, although the effect size was smaller than in riffles. When considered by size-class, the PI index was positive for small and medium sizes and negative for the largest size-class on tiles. The PI index was also generally larger in riffles than in pools on both substrata, and fish predation effects were also generally smaller on gravel than on tile. The only apparent effect of fish on total invertebrate abundance on gravel substrate was a statistically nonsignificant increase in abundance of the smallest size-class in riffles.

Forty-nine percent of the variance in total invertebrate abundance on tile was due to the fish treatment ($F_{1,4} = 14.3$, $P = 0.02$) whereas none of the variance on gravel was accounted for by fish predation ($F_{1,4} = 0.39$, $P = 0.57$). In contrast, 59% of the variance in invertebrate abundance on gravel was due to correlation with organic matter abundance (measured as ash-free dry weight) both within (12%; $F_{1,43} = 19.0$, $P < 0.001$) and between (47%; $F_{443} = 72.7$, $P < 0.001$) enclosures. A much smaller but significant proportion of variance in abundance on tile was also correlated with organic matter both within (6%; $F_{1,33} = 11.7$, $P = 0.005$) and between (17%; $F_{1,33} = 33.9$, $P < 0.001$) enclosures.

Predation effects on individual taxa by habitat and substrate

The proportion of variation in invertebrate abundance that was explained by fish predation was highest for those taxa that appeared to be partly or largely herbivorous, either as grazers (*Ameletus*, *Baetis*) or collector-gatherers on upper substrate surfaces (Orthocladiinae) (Fig. 6). Fish predation had a significant impact on density (evaluated using a sequential Bonferroni correction) only for those taxa marked with an asterisk in Fig. 6: *Ameletus* (split-plot ANOVA, Table 3) and *Zapada* (split-plot ANOVA, $F_{1,4} = 84.4$, $P = 0.008$, $n = 20$) on tile and gravel and orthoclads on tile ($F_{1,4} = 27.8$, $P = 0.006$, $n = 10$). Most other taxa had a relatively minor proportion of variation in abundance accounted for by the presence of fish. PI was negative for larger-bodied mayflies such as *Ameletus* and *Baetis* (Fig. 7) but tended to be positive for smaller taxa such as *Zapada* (Plecoptera: Nemouridae) and orthoclad chironomids, indicating a substantial increase in abundance in fish treatments. Although reduction of *Baetis* density by fish ($P = 0.08$ in all habitats, $P = 0.04$ in riffles) was not significant at a Bonferroni-adjusted level of significance, this is possibly due in part to a lack of statistical power rather than to an absence of predator impact, since density of both *Baetis* and *Ameletus* was significantly reduced in a replicated predation experiment the following year (Rosenfeld 1997*a*). In contrast with *Baetis* and *Ameletus*, heptageniid density (primarily *Cinygmula*) was not strongly affected by the presence of fish. As previously observed by Allan et al. (1986), slight observer movement stimulated an escape response in grazing heptageniids (J. Rosenfeld, personal observation) whereas *Ameletus* has a much higher tolerance to visual stimulation.

Table 2. Results of split-plot ANOVA for the effect of fish predation and enclosure on total invertebrate abundance (log transformed) on tile and gravel substrate.

Substrate	Source	df	SS	MS	*F*	*P*
Tile						
Main effect	Enclosure	4	1.090	0.273	5.77	0.06
	Fish	1	0.234	0.234	4.95	0.09
	Main plot error[a]	4	0.189	0.047	2.23	0.11
Subplot effect	Size	2	7.508	3.754	176.97	0.0001**
	Fish × size	2	0.396	0.198	9.34	0.002**
	Subplot error[b]	16	0.339	0.0212		
Gravel						
Main effect	Enclosure	4	0.391	0.098	10.48	0.022*
	Fish	1	0.000	0.000	0	0.99
	Main plot error[a]	4	0.037	0.009	0.26	0.9
Subplot effect	Size	2	5.527	2.764	76.28	0.0001**
	Fish × size	2	0.012	0.006	0.16	0.85
	Subplot error[b]	16	0.580	0.036		

Note: *Significant at $\alpha = 0.05$; **significant at $\alpha = 0.01$.

[a] Main plot error = (enclosure × fish).

[b] Subplot error = enclosure × substrate + enclosure × fish × substrate).

Table 3. Results of split-plot ANOVA for the effect of fish predation on abundance of *Ameletus* (log transformed).

Effect	Source	df	SS	MS	*F*	*P*
Main	Enclosure	4	0.647	0.162	7.16	0.041*
	Fish	1	0.787	0.787	34.82	0.004**
	Main plot error[a]	4	0.090	0.023	0.87	0.52
Subplot	Substrate	1	0.176	0.176	6.8	0.03*
	Fish × substrate	1	0.087	0.087	3.38	0.1
	Subplot error[b]	8	0.207	0.026		

Note: *Significant at $\alpha = 0.05$; **significant at $\alpha = 0.01$.

[a] Main plot error = (enclosure × fish).

[b] Subplot error = enclosure × substrate + enclosure × fish × substrate).

The relative proportion of variance in invertebrate abundance explained by fish predation was consistently higher on tile substrate than on gravel (Fig. 6), but the taxa most influenced by fish predation were the same on both substrata. In general, the PI index tended to be higher on tile substrate than on gravel (ANOVA for substrate effects on PI indices for all taxa, $F_{1,7} = 6.7$, $P = 0.036$). PI was significantly higher in riffles than in pools for tile substrate (ANOVA, $F_{1,7} = 7.0$, $P = 0.033$) (Fig. 7), but there was no significant habitat effect for gravel.

The relative proportion of variance in abundance of individual taxa that was explained by variance in organic matter within and between enclosures differed among taxa. Detritivores (e.g., tipulids, Tanytarsini chironomids) tended to have a positive relationship with organic matter both within and between enclosures (Fig. 6), although some detritivores (e.g., *Despaxia* sp.) had no significant relationship with the abundance of organic matter at either scale. In general, more of the variance in abundance of most species on gravel substrate was explained by variance in organic matter than by the presence of fish. In contrast, variance in abundance of most taxa on tiles was poorly explained by variance in organic matter, with the exception of Tanytarsini and Tanypodinae chironomids (Fig. 6). Variance in abundance of algivorous invertebrates was largely unrelated to variance in organic matter within enclosures but was sometimes negatively related to variance in organic matter between enclosures for several taxa (*Ameletus*, *Baetis*, and heptageniids) (Fig. 6), indicating a negative association between these grazers and depositional habitats.

Discussion

As predicted, predator effects on different taxa appeared to be strongly related to their feeding habits. Invertebrates that appeared to function as epibenthic grazers or herbivores (large-bodied *Ameletus* and *Baetis*) experienced the largest impact of fish predation. Although absence of a fish effect on heptageniid mayflies suggests differential adaptation of some mayfly taxa to fish predation, decreased grazer density appeared to cause an increase in algal biomass (trophic cascade) in the presence of fish in riffles but not in pools. At the same time, there was an increase in the total abundance of smaller invertebrates in the fish treatments, primarily orthoclad chironomids, which was most pronounced in riffle habitat and on tile substrate.

The increase in abundance of smaller invertebrates in the presence of fish suggests competitive release through predation effects on larger grazers. In separate grazing experiments with *Ameletus* in experimental troughs in Mayfly Creek (Rosenfeld 1997*b*), total invertebrate and orthoclad abundance increased in control treatments without large mayflies and declined in the presence of large grazers, presumably through a combination of exploitative competition for algae and direct consumption of small-instar chironomids. The significant increase in abundance of nemourid stoneflies in the presence of fish suggests the same indirect effect. The possibility that *Zapada* (classified by Merritt and Cummins (1984) as a shredder) are partly herbivorous or otherwise dependent on algal carbon is supported by their much higher densities in riffles than in pools (854 ± 404 versus 279 ± 166·m^{-2}, respectively) and by previous reports of strong algal isotopic signatures for nemourid stoneflies (Rosenfeld and Roff 1992).

Fig. 6. Components of variance of total invertebrate abundance on tile and gravel substrate (pool and riffle habitats combined) related to fish predation (solid bars) and variance in organic matter within (open bars) and between (stippled bars) enclosures. Asterisks indicate significance at α = 0.05 with a sequential Bonferroni adjustment. Negative signs below asterisks indicate a negative relationship between organic matter and abundance of taxa.

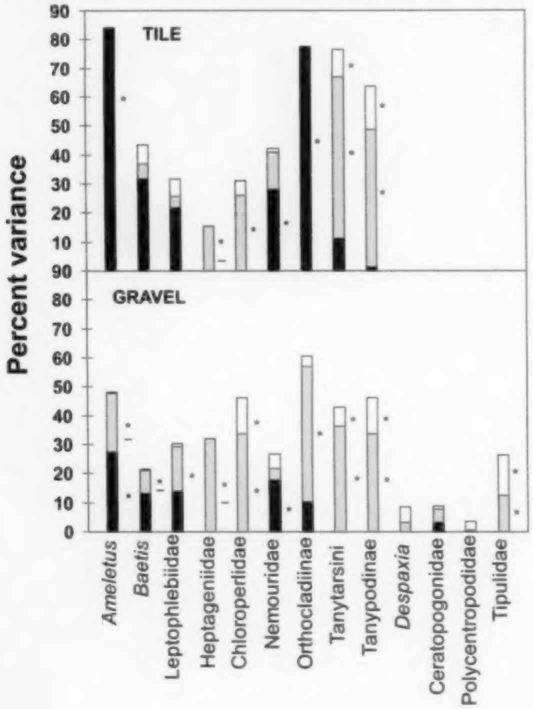
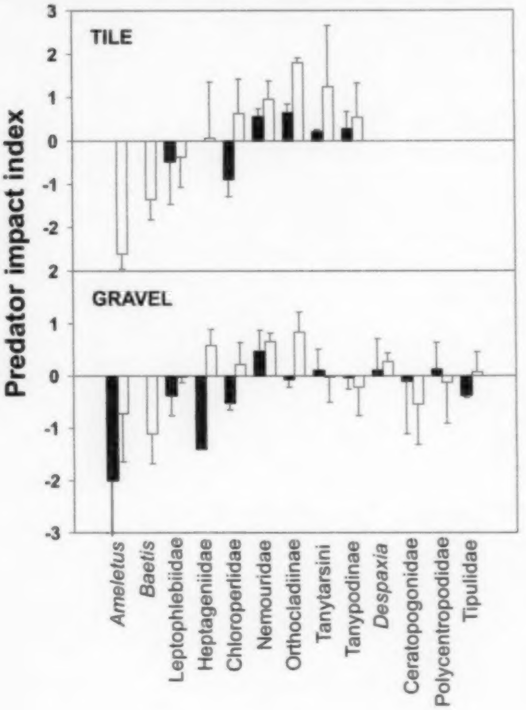


Fig. 7. PI index (±1 SD) for the most common invertebrate taxa on tile and gravel substrate in pool (n = 2) and riffle (n = 3) habitat. Open bars represent riffles and solid bars represent pools.



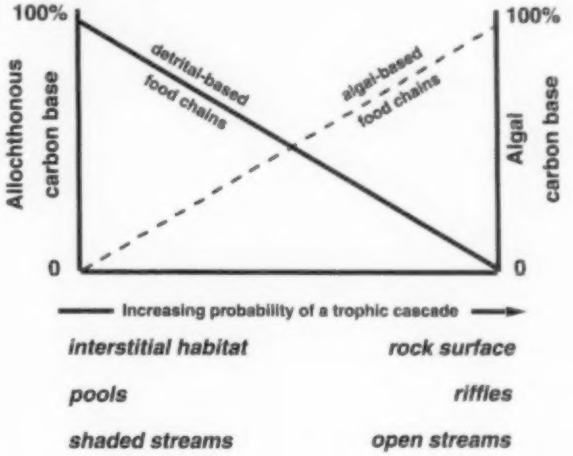
In contrast with the strong effects of fish predation in the primarily algal-based grazing food chain of Mayfly Creek, variance in abundance of detritivores such as Tanytarsini chironomids and tipulids was not significantly influenced by fish predation but was closely related to variance in abundance of their detrital carbon resource. Organic detritus and its associated microflora constitute a carbon resource for many invertebrate taxa in streams, and a general positive relationship between detrital carbon and invertebrate abundance is well documented (e.g., Rabeni and Minshall 1977; Culp et al. 1983). A positive correlation of detritivores with detritus is not unequivocal evidence of resource limitation but is consistent with resource limitation, given that experimental additions of detritus to streams have clearly demonstrated increases in abundance of detritivores (Culp et al. 1983; Richardson 1991). The steeper slope of the invertebrate abundance – organic matter relationship on tile relative to gravel suggests that organic carbon on gravel was lower-quality food relative to that on tile substrate (in terms of fewer invertebrates per gram of organic matter). Lower food quality on gravel is consistent with a lower proportion of periphyton on gravel and higher proportion of coarse particulate organic carbon trapped in gravel interstices, since algal carbon tends to be a higher-quality food resource than woody detritus (Andersen and Cummins 1979; Cummins and Klug 1979).

Consistent with observations in Mayfly Creek, fish effects in algal-based food chains in previous studies commonly result in decreased abundance (e.g., Bechara et al. 1992; Flecker and Townsend 1994; Forrester et al. 1999) or activity (Power 1992*b*; McIntosh and Townsend 1996) of large-bodied herbivores, leading to increased algal biomass and often increased abundance of small epibenthic herbivores. Excretion of nutrients by fish is an alternative hypothesis for increased algal biomass in the presence of fish, but the observed trophic dynamics are most parsimoniously explained by direct fish effects on large grazers. Nevertheless, the role of both predators and herbivores in nutrient cycling in streams is poorly understood and clearly deserves further study. Increased chironomid abundance in the presence of fish has been observed during previous fish predation experiments in both streams (Bechara et al. 1992; Forrester et al. 1999) and lakes (Crowder and Cooper 1982) and appears to be an indirect effect of size-selective fish predation on larger-bodied herbivores. This is supported by independent grazing experiments, which have shown that large grazers reduce the abundance of smaller epibenthic herbivores (e.g., Harvey and Hill 1991; Lamberti et al. 1992; Wooton et al. 1996), primarily chironomids. The generality of this indirect effect of fish predation is supported by a recent metaanalysis of predation effects in streams, which found that chironomids were the only taxa to significantly increase in abundance in the presence of drift-feeding fish (Englund et al. 1999).

Although all three effects (decreased large grazer abundance, increased algal biomass, and abundance of small herbivores) do not consistently occur in all predation experiments, they recur frequently enough to indicate some generality to the underlying mechanisms in simple three-link (sensu Fretwell 1987) algal-based food chains in streams. Interestingly, these effects of fish predation in algal-based food chains at the within-stream scale are also apparent between streams that differ in predation intensity. Comparison of two New Zealand streams that differ in the effectiveness of their fish predators (brown trout versus native galaxis) shows a similar pattern: increased algal biomass and chironomid abundance in the presence of the more effective predator (brown trout; Huryn 1998). There is also some evidence that this pattern appears across a wider landscape scale between fishless and fish-bearing streams. A comparison of benthic community structure between Mayfly Creek and a nearby fish-bearing stream demonstrated greater abundance of algae and small epibenthic invertebrates in the fish-bearing stream (Rosenfeld 2000). Similarly, Harvey (1993), in a broad survey of Utah streams, found fish-bearing streams to have fewer large grazers and increased abundance of small epibenthic herbivores relative to fishless ones. Although Power (1990) also observed strong effects of fish predation in a four-link (sensu Fretwell 1987) algal-based food chain, the presence of an effective invertebrate predator trophic level resulted in a decrease in algal biomass in the presence of fish rather than the increase observed in Mayfly Creek and elsewhere.

As expected, predation effects were greatest on simple substrates (tiles) with minimal refuges. The role of substrate complexity in decreasing predator impact by providing interstitial refuges has been experimentally demonstrated in a number of stream studies (e.g., Brusven and Rose 1981; but see Dahl and Greenberg (1998) for contrasting results). Power (1992*b*) also found that predation effects in algal-based food chains were most pronounced in simple boulder–bedrock habitats and minimal in gravel habitats with abundant interstitial refuges. However, the presence of refuges is confounded by the carbon base of the food chain available to invertebrates on a substrate. Interstitial spaces provide refuges for surface grazers but also accumulate detritus and detritivores, so that as refuges increase, the average carbon base of the food chain on a substrate shifts from algal to detrital. While carbon base and availabilty of refuges appear confounded, they probably function separately to influence vulnerability of invertebrates to fish. Predation effects were greater in riffles than in pools in Mayfly Creek, independent of substrate type and the availability of refuges, which is consistent with the hypothesis that predation effects are most pronounced when the carbon base of a substrate is predominantly algal rather than detrital.

Fig. 8. Schematic illustrating the relationship between habitat type, carbon source, and the predicted strength of trophic interactions in streams.



As a consequence of deposition in gravel interstices, detrital carbon was roughly two orders of magnitude higher on gravel substrate than on tile. At the channel unit scale, detrital carbon was also on average three to five times higher in pools than in riffles. In contrast, algal biomass differed little between pools and riffles; subsequent chlorophyll *a* extraction on tile substrate also indicated little difference in algal biomass between gravel and tile (Rosenfeld 1997*a*), although algal biomass on tile was not directly measured in this study. This pattern of algal and detrital carbon distribution indicates accumulation of detritus with increasing substrate complexity and decreasing current velocity and a consequent reduction in the relative contribution of algal carbon to the local carbon base. If trophic cascades are more pronounced in algal-based food chains, then the local effects of fish predation on the entire benthic community should become weaker as predation effects in the epibenthos are diluted (Bechara et al. 1993) by inclusion of interstitial detritus and less vulnerable detritivores (Fig. 8). At a larger spatial scale, one might hypothesize that predation effects on the entire benthic community may become more pronounced as light limitation decreases, and increases in algal production shift the food base away from interstitial detritus towards epibenthic production, causing a greater proportion of the invertebrate community to become exposed to fish predation (Fig. 8).

Algal- and detrital-based food chains are a useful construct for understanding trophic interactions in streams and have additional features beyond differences in the vulnerability of primary consumers that will influence consumer–resource dynamics. Feedback between invertebrate consumers and their periphyton resource should be strong in algal-based food chains because grazers have the potential to reduce both the biomass and the production of the primary producer trophic level (e.g., Lamberti and Moore 1984). Detritivorous food chains, however, are donor controlled (Richardson 1993; Polis and Strong 1996). Detritivores have the potential to reduce the biomass of their detrital resource, but they can have no effect on its rate of production, which is determined by allochthonous inputs from the riparian zone or detrital production from algal-based food chains. A better understanding of the linkages between parallel algal- and detrital-based food chains is critical, since food chains are rarely independent and "multichannel omnivory" (e.g., feeding on both algae and detritus) may influence the strength of trophic interactions in a focal food chain (Polis and Strong 1996). This is especially true in open systems such as streams, where the effects of fish predation in algal-based food chains may be enhanced by a predator trophic level that is subsidized through inputs of aquatic invertebrates from detrital-based food chains or by terrestrial invertebrates falling onto the stream surface.

Although trophic cascades appear to be rare in aquatic detrital food chains (Polis and Strong 1996), invertebrate predators may be able to forage more effectively in interstices and detritus than fish, with the potential for a trophic cascade (e.g., Oberndorfer et al. 1984). Detritivores may also in some instances be significantly affected by fish predation; in particular, freshwater gammarids (e.g., Friberg et al. 1994) and isopods (Holomuzki and Hatchett 1994) appear to both be vulnerable to fish predation and have the potential to measurably affect detrital abundance (Short and Holomuzki 1992). Consequently, their presence may significantly alter overall fish–invertebrate–resource dynamics. Similarly, the presence of algivorous or detritivorous fish in tropical streams (Flecker 1992) may have more pervasive effects on benthic community structure than the direct effects of generalized insectivores more typical of north temperate streams with histories of recent glaciation.

Physical habitat structure clearly has a pivotal influence on biotic processes in streams, ranging from community structure to production processes (Huryn and Wallace 1987). The present study indicates that variation in the strength of fish effects can also be constructively understood within the context of the physical stream habitat and its influence on the local carbon base in streams.

Acknowledgements

I would like to acknowledge the able assistance of Bill Graham, Daysun Wrubel, and Ryan Stevenson in the field as well as a host of volunteers from the ecology group at the University of British Columbia. The staff at the Malcolm Knapp Research Forest also provided indispensable support. Ernest Keeley, John Richardson, Eric Taylor, Mike Whitlock, Ralph Yorque, and especially Lorne Rothman and Dolph Schluter provided helpful statistical advice, and the manuscript was greatly improved by comments from several anonymous reviewers. William E. Neill contributed astute advice and support throughout the course of this study, which was funded by a Natural Sciences and Engineering Research Council of Canada operating grant to William E. Neill and a Natural Sciences and Engineering Research Council of Canada postgraduate fellowship to J.R.

References

Allan, J.D. 1978. Trout predation and the size composition of stream drift. Limnol. Oceanogr. **23**: 1231–1237.

Allan, J.D. 1981. Determinants of diet of brook trout (*Salvelinus fontinalis*) in a mountain stream. Can. J. Fish. Aquat. Sci. **38**: 184–192.

Allan, J.D. 1982. The effects of reduction in trout density on the invertebrate community in a mountain stream. Ecology, **63**: 1444–1455.

Allan, J.D. 1984. The size composition of invertebrate drift in a Rocky Mountain stream. Oikos, **42**: 68–76.

Allan, J.D., Flecker, A.S., and McClintock, N.L. 1986. Diel epibenthic activity of mayfly nymphs, and its non-concordance with drift. Limnol. Oceanogr. **31**: 1057–1065.

Andersen, N.H., and Cummins, K.W. 1979. Influence of diet on the life histories of aquatic insects. J. Fish. Res. Board Can. **36**: 335–342.

Bechara, J.A., Moreau, G., and Planas, D. 1992. Top-down effects of brook trout (*Salvelinus fontinalis*) in a boreal forest stream. Can. J. Fish. Aquat. Sci. **49**: 2093–2103.

Bechara, J.A., Moreau, G., and Hare, L. 1993. The impact of brook trout (*Salvelinus fontinalis*) on an experimental stream benthic community: the role of spatial and size refugia. J. Anim. Ecol. **62**: 451–464.

Brooks, J.L., and Dodson, S. 1965. Predation, body size, and competition of plankton. Science (Washington, D.C.), **150**: 28–35.

Brusven, M.A., and Rose, S.T. 1981. Influence of substrate composition and suspended sediment on insect predation by the torrent sculpin, *Cottus rhotheus*. Can. J. Fish. Aquat. Sci. **38**: 1444–1448.

Cooper, S.D., Walde, S.J., and Peckarsky, B.L. 1990. Prey exchange rates and the impact of predators on prey populations in streams. Ecology, **71**: 1503–1514.

Crowder, L.B., and Cooper, W.E. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology, **63**: 894–908.

Culp, J.M. 1986. Experimental evidence that stream macroinvertebrate community structure is unaffected by different densities of coho salmon fry. J. North Am. Benthol. Soc. **5**: 140–149.

Culp, J.M., Walde, S.J., and Davies, R.W. 1983. Relative importance of substrate particle size and detritus to stream benthic macroinvertebrate microdistribution. Can. J. Fish. Aquat. Sci. **40**: 1568–1574.

Cummins, K.W., and Klug, M.J. 1979. Feeding ecology of stream invertebrates. Annu. Rev. Ecol. Syst. **10**: 147–172.

Dahl, D., and Greenberg, L. 1996. Impact on stream benthic prey by benthic vs. drift feeding predators: a meta-analysis. Oikos, **77**: 177–181.

Dahl, J., and Greenberg, L.A. 1998. Effects of fish predation and habitat type on stream benthic communities. Hydrobiologia, **361**: 67–76.

Englund, G. 1997. Importance of spatial scale and prey movements in predator caging experiments. Ecology, **78**: 2316–2325.

Englund, G., Sarnelle, O., and Cooper, S.D. 1999. The importance of data selection criteria: meta-analyses of stream predation experiments. Ecology, **80**: 1132–1141.

Flecker, A.S. 1992. Fish trophic guilds and the structure of a tropical stream: weak direct vs. strong indirect effects. Ecology, **73**: 927–940.

Flecker, A.S., and Allan, J.D. 1984. The importance of predation, substrate, and spatial refugia in determining lotic insect distributions. Oecologia, **64**: 306–313.

Flecker, A.S., and Townsend, C.R. 1994. Community-wide consequences of trout introduction in New Zealand streams. Ecol. Appl. **4**: 798–807.

Forrester, G.E., Dudley, T.L., and Grimm, N.B. 1999. Trophic interactions in open systems: effects of predators and nutrients on stream food chains. Limnol. Oceanogr. **44**: 1187–1197.

Fretwell, S.D. 1987. Food chain dynamics: the central theory of ecology? Oikos, **50**: 291–301.

Friberg, N., Andersen, T.H., Hansen, H.O., Iversen, T.M., Jacobsen, D., Krojgaard, L., and Larsen, S.E. 1994. The effect of brown trout (*Salmo trutta* L.) on stream invertebrate drift, with special reference to *Gammarus pulex* L. Hydrobiologia, **294**: 105–110.

Harvey, B.C. 1993. Benthic assemblages in Utah headwater streams with and without trout. Can. J. Zool. **71**: 896–900.

Harvey, B.C., and Hill, W.R. 1991. Effects of snails and fish on benthic invertebrate assemblages in a headwater stream. J. North Am. Benthol. Soc. **10**: 263–270.

Holomuzki, J.R., and Hatchett, L.A. 1994. Predator avoidance costs and habituation to fish chemicals by a stream isopod. Freshwater Biol. **32**: 585–592.

Hunter, M.D., and Price, P.W. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology, **73**: 724–732.

Huryn, A. 1998. Ecosystem-level evidence of top-town and bottom-up control of production in a grassland stream system. Oecologia, **115**: 173–183.

Huryn, A., and Wallace, B.J. 1987. Local geomorphology as a determinant of local macrofaunal production in a mountain stream. Ecology, **68**: 1932–1942.

Kohler, S.L., and McPeek, M.A. 1989. Predation risk and the foraging behavior of competing stream insects. Ecology, **70**: 1811–1825.

Lamberti, G.A., and Moore, J.W. 1984. Aquatic insects as primary consumers. *In* The ecology of aquatic insects. *Edited by* V.H. Resh and D.M. Rosenberg. Prager Science, New York. pp. 164–195.

Lamberti, G.A., and Resh, V.A. 1985. Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae, and macroinvertebrates. Freshwater Biol. **15**: 21–30.

Lamberti, G.A., Gregory, S.V., Hawkins, C.P., Wildman, R.C., Ashkenas, L.R., and DeNicola, D.M. 1992. Plant–herbivore interactions in streams near Mount St. Helens. Freshwater Biol. **27**: 237–247.

McIntosh, A.R., and Townsend, C.R. 1996. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behaviour? Oecologia, **108**: 174–181.

Merritt, R.W., and Cummins, K.W. 1984. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa.

Neill, W.E. 1994. Spatial and temporal scaling and the organization of limnetic communities. *In* Aquatic ecology: scale, pattern, and process. *Edited by* P.S. Giller, A.G. Hildrew, and D.G. Raffaelli. Blackwell Scientific Publications, Oxford, U.K. pp. 189–231.

Oberndorfer, R.Y., McArthur, J.V., Barnes, J.R., and Dixon, J. 1984. The effect of invertebrate predators on leaf litter processing in an alpine stream. Ecology, **65**: 1325–1331.

Polis, G.A., and Strong, D.R. 1996. Food web complexity and community dynamics. Am. Nat. **147**: 813–846.

Power, M.E. 1990. Effects of fish in river food webs. Science (Washington, D.C.), **250**: 811–814.

Power, M.E. 1992*a*. Top-down and bottom-up forces in food webs: do plants have primacy? Ecology, **73**: 733–746.

Power, M.E. 1992*b*. Habitat heterogeneity and the functional significance of fish in river food webs. Ecology, **73**: 1675–1688.

Rabeni, C.F., and Minshall, G.W. 1977. Factors affecting the microdistribution of stream benthos. Oikos, **29**: 33–43.

Richardson, J.S. 1991. Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology, **72**: 873–887.

Richardson, J.S. 1993. Limits to productivity in streams: evidence from studies of macroinvertebrates. *In* Production of juvenile Atlantic salmon, *Salmo salar*, in natural waters. *Edited by* R.J. Gibson and R.E. Cutting. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 9–15.

Rosenfeld, J.S. 1997*a*. The influence of upstream predation on the expression of fish effects in downstream patches. Freshwater Biol. **37**: 535–543.

Rosenfeld, J.S. 1997*b*. The effect of large macroinvertebrate herbivores on sessile epibenthos in a mountain stream. Hydrobiologia, **344**: 75–79.

Rosenfeld, J.S. 2000. Contrasting effects of fish predation in a fishless and fish-bearing stream. Arch. Hydrobiol. **147**: 129–142.

Rosenfeld, J.S., and Hudson, J.J. 1997. Primary production, bacterial production, and invertebrate biomass in pools and riffles in southern Ontario streams. Arch. Hydrobiol. **139**: 301–316.

Rosenfeld, J.S., and Roff, J.C. 1992. Examination of the carbon base in southern Ontario streams using stable isotopes. J. North Am. Benthol. Soc. **11**: 1–10.

Short, T.M., and Holomuzki, J.R. 1992. Indirect effects of fish on foraging behaviour and leaf processing by the isopod *Lirceus fontinalis*. Freshwater Biol. **27**: 91–97.

Smock, L.A. 1980. Relationships between body size and biomass of aquatic insects. Freshwater Biol. **10**: 375–383.

Snedecor, G.W., and Cochran, W.G. 1989. Statistical methods. 8th ed. Iowa State University, Ames, Iowa.

Sokal, R.R., and Rohlf, F.J. 1997. Biometry. Freeman and Company, New York.

Wooster, D. 1994. Predator impacts on stream benthic prey. Oecologia, **99**: 7–15.

Wooton, J.T., Parker, M.S., and Power, M.E. 1996. Effects of disturbance in river food webs. Science (Washington, D.C.), **273**: 1558–1561.

Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes

Barry J.F. Biggs, Steven N. Francoeur, Alexander D. Huryn, Roger Young, Christopher J. Arbuckle, and Colin R. Townsend

Abstract: We tested the hypothesis that differences in top-level predators could mediate the importance of top-down versus bottom-up forces in stream food chains using three streams dominated by *Galaxias* (fish native to New Zealand) and three dominated by brown trout (*Salmo trutta*) (an introduced species). These two fish species have quite different predation strategies and energetic requirements. Periphyton in the *Galaxias* streams formed relatively low biomass (indicative of high invertebrate grazing), whereas periphyton in two of the three trout streams formed relatively high biomass (indicative of lower grazing pressure). Periphyton biomass response to inorganic nutrient enrichment varied by fish type. Invertebrate densities on artificial substrates were higher in the *Galaxias* streams, with the exception of chironomids. Nutrient enrichment of periphyton led to increases in total invertebrate densities, but these increases were greater in the *Galaxias* streams where invertebrate predation was thought to be lower. Our results suggest that the specific feeding behavior traits of the consumers should be considered when investigating trophic cascades or predicting effects of bottom-up nutrient enrichment on autotrophy.

Résumé : Nous sommes partis de l'hypothèse que des différences dans les prédateurs supérieurs pourraient agir sur l'importance relative des forces descendantes et des forces ascendantes dans les chaînes trophiques des milieux lotiques en étudiant trois cours d'eau dominés par *Galaxias* (poisson indigène de la Nouvelle-Zélande) et trois cours d'eau dominés par la truite brune (espèce introduite). Chez ces deux poissons, les stratégies de prédation et les besoins énergétiques sont très différents. Dans les cours d'eau à *Galaxias*, le périphyton formait une biomasse relativement faible (ce qui indique un fort broutage par les invertébrés), tandis que, dans deux des trois cours d'eau à truite brune, le périphyton formait une biomasse relativement élevée (ce qui indique une faible pression de broutage). La réaction de la biomasse de périphyton à un enrichissement en nutriants inorganiques variait selon le type de poisson. Les densités d'invertébrés sur les substrats artificiels étaient plus élevées dans les cours d'eau à *Galaxias*, à l'exception des chironomidés. L'enrichissement nutritif du périphyton a amené des augmentations des densités totales d'invertébrés, mais ces augmentations étaient plus fortes dans les cours d'eau à *Galaxias*, où la prédation par les invertébrés était jugée plus faible. Nos résultats permettent de penser que les traits spécifiques du comportement alimentaire des consommateurs doivent être considérés lorsqu'on étudie les cascades trophiques ou lorsqu'on veut prédire les effets sur l'autotrophie de l'enrichissement en nutriants à partir de la base de la chaîne trophique.

[Traduit par la Rédaction]

Introduction

Ecologists have long recognized that the effects of predators ("top-down" forces) and resources ("bottom-up" forces) on biomass will vary among trophic levels (e.g., Oksanen et al. 1981; Power 1992; Nisbet et al. 1997). A well-known example of top-down effects in marine ecosystems is the dramatic increase recorded in grazing sea urchin populations due to release from predation pressure in some regions that have been overfished. This has then resulted in extensive overgrazing of benthic algae by sea urchins, turning these areas into coralline barrens (Elner and Vadas 1990; Sala et

Received November 16, 1999. Accepted March 24, 2000.
J15442

B.J.F. Biggs[1] and S.N. Francoeur.[2] National Institute of Water and Atmospheric Research Ltd. (NIWA), P.O. Box 8602, Christchurch, New Zealand.
A.D. Huryn,[3] R. Young,[4] C.J. Arbuckle, and C.R. Townsend. Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand.

[1] Author to whom all correspondence should be addressed. e-mail: b.biggs@niwa.cri.nz
[2] Present address: Department of Biology, University of Alabama, Tusculoosa, AL 35486, U.S.A.
[3] Present address: Department of Biological Sciences, 5722 Deering Hall, University of Maine, Orono, ME 04469-5722, U.S.A.
[4] Present address: Cawthron Institute, Private Bag 2, Nelson, New Zealand.

al. 1998). Conversely, many experiments (particularly in streams and lakes) have shown how nutrient addition can stimulate autotrophic production and associated invertebrate herbivores (e.g., McQueen et al. 1989; Biggs and Lowe 1994; Brett and Goldman 1997). Such observations, and many earlier studies from other habitats, have led to the development of a number of conceptual and mathematical models to represent this "trophic cascading" (e.g., Power 1992). Generally, these models predict strong response of autotrophic production to nutrient enrichment in odd-link systems where herbivores are controlled by predators and no significant response in even-link systems where the density of herbivores is largely unconstrained by predators.

However, there are several areas where our knowledge of trophic cascading is still relatively weak. First, few studies have investigated the simultaneous influence of predators and nutrients in open-ended three-level trophic systems so that the relative importance of their effects on ecosystem functioning can be directly compared (Nisbet et al. 1997; Stiling and Rossi 1997; Forrester et al. 1999). Second, reviews of forces controlling trophic interactions have suggested that heterogeneity in physical and biotic factors might mediate trophic interactions and resultant cascading, but little is known of such effects (Hunter and Price 1992; Power 1992). The relative strength of top-down versus bottom-up forces in food webs could partly depend on the efficiency with which consumers can exploit their prey and top-down forces could be weakened relative to bottom-up forces by factors that reduce consumer efficiency (Power 1992). Indeed, Hunter and Price (1992) posited that "the identities of individual species and environmental variation are as important determinants of population and community dynamics as are the number of levels in a food chain or the position of the system along a resource gradient."

Brown trout (*Salmo trutta*) were first introduced into New Zealand in the late 1880s and interact strongly with native fish (mainly species in the family Galaxiidae) to the extent that these trout have replaced *Galaxias* spp. as the main vertebrate predator in many streams (Crowl et al. 1992). Recent studies have indicated that this shift in dominant predator has introduced a significant change in predation regime for herbivores in New Zealand streams. First, brown trout tend to attain much higher biomass and be physically larger per individual than native *Galaxias* (e.g., Huryn 1998). Consequently, consumption of grazing invertebrates to sustain bioenergetic requirements is expected to be much greater in trout-dominated streams. Second, there are significant behavioral differences between brown trout and *Galaxias* that are known to influence grazing activity by invertebrates. *Galaxias* forage openly on benthos and terrestrial prey at night using mechanical cues (i.e., the prey items need to be moving to be located) and seek refuge in the substrates during the day (although they still attack prey from such positions if the prey stray within range; McIntosh and Townsend 1995*a*). Conversely, brown trout are visual feeders and forage mainly during the day and are more aggressive predators (McIntosh and Townsend 1994, 1995*a*). Such differences in strategy have been found to result in major changes in grazer behavior, with mayfly herbivores grazing openly on the surface of stones during the day in *Galaxias* streams, but with grazing largely restricted to the night in trout streams (McIntosh and Townsend 1994, 1995*a*). In experiments conducted in artificial channels, Flecker and Townsend (1994) found that the presence of either trout or *Galaxias* resulted in a reduction in grazing by invertebrates and a subsequent increase in periphyton biomass, but with trout exerting a significantly stronger effect relative to *Galaxias*. The above results suggest that even with three trophic levels, the cascading effect to lower trophic levels could be highly variable and not consistent with current models of trophic cascading, depending on the characteristics of the top predator.

To be conclusive about the stream-wide importance of heterogeneity in predators and predation strategies, we need more comprehensive analyses of relative autotrophic and consumer responses to different predators. Ideally, such studies should be conducted in natural trilevel systems where fish and invertebrates are free to feed, roam, and seek refuge at their will and accrual of autotrophic biomass reflects a true balance between the sum of natural gain and loss processes. Autotrophs are especially vulnerable in trophic cascades (Strong 1992) and may be the best barometer of differences in predation effects.

In the following study, we investigate whether different top aquatic predator feeding strategies mediate different responses in autotrophic and consumer production to nutrient enrichment. Six streams were used for the study, three dominated by *Galaxias* and three dominated by trout. We hypothesized that lower predation pressure may result in higher grazer densities and lower periphyton biomass in *Galaxias* streams than in trout streams, whereas there would be a weaker response by grazers but a higher response by periphyton to bottom-up inorganic enrichment in trout streams than in *Galaxias* streams because of higher predation pressure in the trout streams. Indeed, are trout streams greener than *Galaxias* streams?

Materials and methods

Study area and streams

The study streams were all in the Taieri River drainage basin, southern South Island, New Zealand (Fig. 1). Surveys of the Taieri River tributaries had defined that many streams were largely dominated by trout or *Galaxias* (McIntosh and Townsend 1995*a*, 1995*b*), and we used this as a basis for site selection. Three pairs of trout and *Galaxias* streams were chosen within this drainage basin (Fig. 1; Table 1). Each pair of streams (i.e., a *Galaxias* and a trout stream) was chosen to be physically adjacent to minimize local variations in temperature, nutrients, and substrate type. The catchments of all streams were underlain by highly metamorphosed sediments (Permian to Carboniferous chlorite schist) and had a vegetation cover of tall native tussock grass (*Chionochloa* spp.) oversown with pasture grasses. This vegetation was lightly grazed by sheep and cattle.

An extended period of summer low flows (February 1995) was specifically chosen for this analysis so that biotic interactions could become more fully developed, thus avoiding the confounding effects of flood disturbance. Each site was located in a run. These habitats comprised relatively uniform, low-gradient sections with an unbroken water surface, approximately 50 m in length with depths of 0.1–0.2 m and velocities of 0.2–0.4 $m \cdot s^{-1}$. Bed sediments at all sites were dominated by cobble- and boulder-sized particles, with bedrock usually being subdominant. All stream waters were very clear (turbidity <2 nephelometric turbidity units) with rela-

Table 1. Physical, water quality (±SE), and fish characteristics of sampling sites.

Site	Region	Fish	Fish density (fish·m^{-2})	Fish biomass (g·m^{-2})	Relative abundance of prostrate periphyton	Relative abundance of erect periphyton
Healy Creek	1	G	4.75	3.23	7.0 (0.00)	6.65 (0.35)
Kyeburn Stream	1	T	0.17	4.2	5.3 (1.00)	6.5 (0.20)
Unnamed creek	2	G	1.32	0.002	4.2 (1.15)	4.3 (0.55)
McHardies Creek	2	T	2.40	7.56	0 (0.00)	6.0 (0.00)
Stony Creek	3	G	2.14	0.74	3.5 (0.50)	4.9 (0.10)
Sutton Stream	3	T	2.26	12.1	2.50 (0.50)	5.8 (0.25)

Note: Relative abundance of periphyton taxa is based on a 0–8 scale of biovolume. G, *Galaxias*; T, brown trout; P_{max}, maximum primary production; CR, community respiration.

tively low concentrations of nutrients at the time of the study (total P = 5–19 mg·m^{-3}, total N = 73–259 mg·m^{-3}).

Field sampling

We surveyed streambed periphyton and seston concentrations in all streams to establish the background nature of the communities and also in an attempt to detect system-wide differences as a function of fish predation regime. At each site, we established two transects, 2–3 m apart, and retrieved eight cobbles from the bed at equal intervals across the transects. Each set of cobbles was then pooled for an analysis of community metabolism (see below). Following this, the cobbles were returned to the stream bank and all periphyton thoroughly scrubbed from the cobble surfaces in a bucket of water using a coarse bristle plastic brush. The resultant pooled slurry for each transect was then kept on ice for about 7 h when it was frozen until analysis (generally within 2 weeks).

We quantified maximum primary production (P_{max}) and community respiration (CR) at each site using a dissolved oxygen respirometer while the periphyton was still attached to the cobbles. The set of eight cobbles per replicate transect at each site was placed in a plexiglass chamber (internal dimensions = 0.16 × 0.38 m) that was submerged in 0.3 m of flowing water (to maintain ambient temperature) and sealed. A quartz halogen lamp powered by a stream bank generator was then placed on a stand 0.5 m above the chamber to provide a constant light source (giving a light intensity of about 550 μmol·m^{-2}·s^{-1} within the chamber) and a submersible pump inserted in a chamber return line connected to a 12-V DC battery on the stream bank to generate flow in the chamber (about 0.25–0.3 m·s^{-1} depending on substrate roughness in the chamber). A Yellow Springs Instrument dissolved oxygen probe was inserted in the water return line to measure changes in dissolved oxygen over a 10-min incubation period. The signal from the dissolved oxygen meter was routed through an electronic scale expander to subtract the starting oxygen concentration and expand a 1-mg·L^{-1} working range to full scale. Changes in oxygen concentration with a precision of 0.005 mg·L^{-1} were then displayed on a chart recorder. Linear kinetics were generally achieved within 2–5 min of the commencement of each incubation, and the gradient of the chart line was used to calculate maximum net primary production (NPP). Once sufficient record had been obtained, the chamber was covered with a black rubber sheet to determine CR. The P_{max} was calculated as the sum of NPP and CR.

Fish density and biomass were calculated from depletion-removal electric fishing of a 30-m reach in each stream 1 week after the invertebrate and algal sampling was conducted. The feeding function of the fish is derived from extensive previous studies in the area (e.g., Crowl et al. 1992; McIntosh and Townsend 1995*a*, 1996). Benthic invertebrates composed the majority of prey items.

In-stream inorganic nutrient enrichment experiments

The relative response of autotrophy to bottom-up nutrient resource stimulation as a function of predator type was determined using nutrient-diffusing substrates. The substrates consisted of a galvanized steel tray (dimensions = 0.6 × 0.4 × 0.2 m) with an internal frame that held four lines of five 300-mL plastic jars containing nutrient-enriched agar treatments (N, P, N+P, control for each line). Each jar had a neck diameter of 7.5 cm and was capped with a fine crystalline Whatman 542 hardened ashless filter (90 mm in diameter) that was held in place using a tightly fitting plastic collar. These filters were the periphyton (and invertebrate) colonization surfaces. A lid with holes that were the same diameter as the tops of the nutrient agar jars was then fitted to the top of the sampler trays so that the filter sampling surface was flush with the lid and exposed to the overlying water. Partitions running in a streamwise direction, 2 cm high, separated each line of filter paper colonization surfaces. In midstream, the trays were then buried in the bed sediments so that the lid (and filter sampling surfaces) was flush with the surface of the streambed. This enabled full access of invertebrates from the natural bed sediments to the surface of the samplers. The assays were left for a 21-day incubation period in each stream. The nutrient treatments comprised the 300-mL plastic jars filled with 2% agar solution with either 0.05 M Na_2HPO_4 (P), 0.5 M $NaNO_3$ (N), or a mixture of both at the same concentrations (N+P). Agar without nutrients served as the controls. At the end of the incubation the hardened ashless filters were removed for direct extraction of chlorophyll *a*.

To quantify differences in response of invertebrates to inorganic nutrient enrichment between *Galaxias* and trout streams, we enumerated the invertebrates on each of the nutrient-diffusing substrate replicates into broad taxonomic groups (snails, caddisflies, chironomids). Mayflies were counted before the samplers were disturbed. The feeding function of the invertebrates was determined following extensive gut content analysis (e.g., Townsend et al. 1998). The majority (by far) were grazers.

Laboratory analyses

In the laboratory, samples from each transect were thawed, homogenized using a blender, and subsampled for biomass and taxonomic analyses. Three subsamples (each composed of three pooled 5-mL aliquots of sample slurry) were separately filtered and analyzed for each transect. Chlorophyll *a* was extracted from the filters using boiling 90% ethanol with absorbance read at 665 nm on a spectrophotometer (with readings after acidification to correct for phaeopigments). Ash-free dry mass (AFDM) was determined on a separate set of subsamples (obtained as for chlorophyll *a*) by drying at 105°C for 24 h and ashing at 440°C for 4 h. Chlorophyll *a* and AFDM values were normalized for the upper two thirds of surface area of the cobbles using the equation of Biggs and Close (1989) for calculating the surface area of stone colonized by periphyton based on the *x*, *y*, and *z* dimensions of the stones. Percent chlorophyll *a* was determined for the periphyton from these cobbles as chlorophyll *a*/AFDM (both in milligrams per square metre) × 100.

The taxonomic subsamples were enumerated using an inverted microscope at 480× and 780× magnification to qualitatively estimate the dominant taxon based on its contribution to the sample's

Chl *a* ($mg \cdot m^{-2}$)	AFDM ($g \cdot m^{-2}$)	Chl *a* (%)	Organic seston ($g \cdot m^{-3}$)	Inorganic seston ($g \cdot m^{-3}$)	P_{max} (mg $O_2 \cdot m^{-2} \cdot h^{-1}$)	CR (mg $O_2 \cdot m^{-2} \cdot h^{-1}$)	P_{max}:CR
10.0 (0.18)	4.3 (0.57)	0.24 (0.027)	0.56 (0.122)	0.41 (0.110)	0.43 (0.080)	0.45 (0.054)	0.94 (0.065)
9.7 (2.32)	5.7 (1.0)	0.17 (0.012)	0.60 (0.096)	0.55 (0.222)	0.35 (0.033)	0.27 (0.004)	1.26 (0.103)
6.7 (0.48)	9.2 (0.08)	0.07 (0.005)	3.49 (0.396)	3.45 (0.298)	0.93 (0.966)	0.63 (0.438)	0.80 (0.968)
17.9 (0.02)	12.0 (0.66)	0.15 (0.007)	0.77 (0.051)	0.889 (0.058)	1.97 (0.887)	0.46 (0.039)	4.18 (1.584)
1.0 (0.06)	1.95 (0.04)	0.05 (0.003)	1.34 (0.135)	1.17 (0.295)	0.22 (0.176)	0.64 (0.102)	0.39 (0.336)
6.9 (0.85)	3.7 (0.88)	0.19 (0.022)	0.66 (0.058)	0.52 0.071)	0.00 (0.114)	0.56 (0.033)	–0.12 (0.198)

biovolume (i.e., an integration of frequency × size). All other taxa were ranked on an eight-point linear scale based on their biovolume in relation to that of the dominant taxon. If two taxa were codominant, they were both given a score of 8. Generally, one taxon, or at the most three taxa, made up approximately 70% of the communities. Some of the common filamentous taxa could not be identified to species level because essential distinguishing characteristics were not present (e.g., reproductive structures). Since general food chain theory (e.g., Fretwell 1977) predicts that the vulnerability of a taxon to herbivory is positively correlated with its competitive ability (i.e., in the absence of grazing, periphyton assemblages should be dominated by highly competitive but vulnerable taxa; Bronmark et al. 1992), we classified most of the taxa found at the sites into two groups as a general indicator of possible system-wide effects of the different predation regimes. These groups were (*i*) not or only weakly vulnerable to herbivory and (*ii*) vulnerable to herbivory. Taxa classified as not (or only weakly) vulnerable to herbivory were assumed to be prostrate understory taxa and (or) unpalatable taxa (Steinman 1996). In this group, we classified the diatoms *Cocconeis* sp., *Cymbella aspera*, *Epithemia sorex*, and *Epithemia zebra* var. *saxonica* as having low vulnerability to grazing and the cyanobacteria *Nostoc* sp. and *Tolypothrix* sp. as having low palatability. Taxa classified as being vulnerable to herbivores (either directly through grazing or indirectly through physical dislodgment during herbivore browsing; Steinman 1996) were assumed to be erect overstory taxa. In this group we included the stalked diatoms *Gomphoneis herculeana* and *Gomphonema tenellum*, the erect sessile diatom *Synedra ulna*, the fragile chain-forming diatom *Tabellaria flocculosa*; the filamentous green algae *Bulbochaete* spp., *Microspora* spp., and *Oedogonium* sp., and the filamentous red alga *Audouinella hermanii*. The average relative abundance of taxa in each of these two classes was calculated for each streambed transect. We considered that a higher relative abundance of prostrate/unpalatable taxa and a lower relative abundance of erect taxa should indicate a strong influence of herbivores on the communities (Steinman 1996). Conversely, we considered that a relatively high abundance of erect taxa and a relatively low abundance of prostrate/unpalatable taxa should indicate low grazing pressure (Steinman 1996).

To measure organic and inorganic seston concentration at each site, five replicate samples of stream water were collected in 20-L drums. Portions of the sample were then filtered with mild suction through Whatman GF/F filters (pore size about 0.6 μm) that had previously been rinsed, ashed, and weighed. All samples were oven dried (24 h at 60°C), weighed, ashed (1 h at 550°C), rewetted with distilled water, redried, and reweighed. Organic seston (AFDM) and inorganic seston (ash) were calculated and converted to concentration values by dividing by the volume of water filtered in each case.

Data analysis

Possible system-wide effects of fish on streambed periphyton communities and seston were tested as a function of region where the site pair was located and fish predation regime using analysis of variance (ANOVA). Parameters for a two-way model (region and fish effects) were first estimated and the effects of the fish treatment specifically tested using interstream variability as the error term (i.e., the mean squares of the region × fish interaction). The specific effects of the predation regimes on autotrophic and consumer responses to nutrients were tested using split-plot ANOVA. The effects of N, P, fish, and their interactions were determined using the region × fish × N × P interaction residual mean square as the error term for calculating the variance ratios. Probability plots of residuals were analyzed, and where necessary, log $x + 1$ transformations were applied to correct for homogeneity of variances and the analyses rerun.

Results

Streambed periphyton and seston

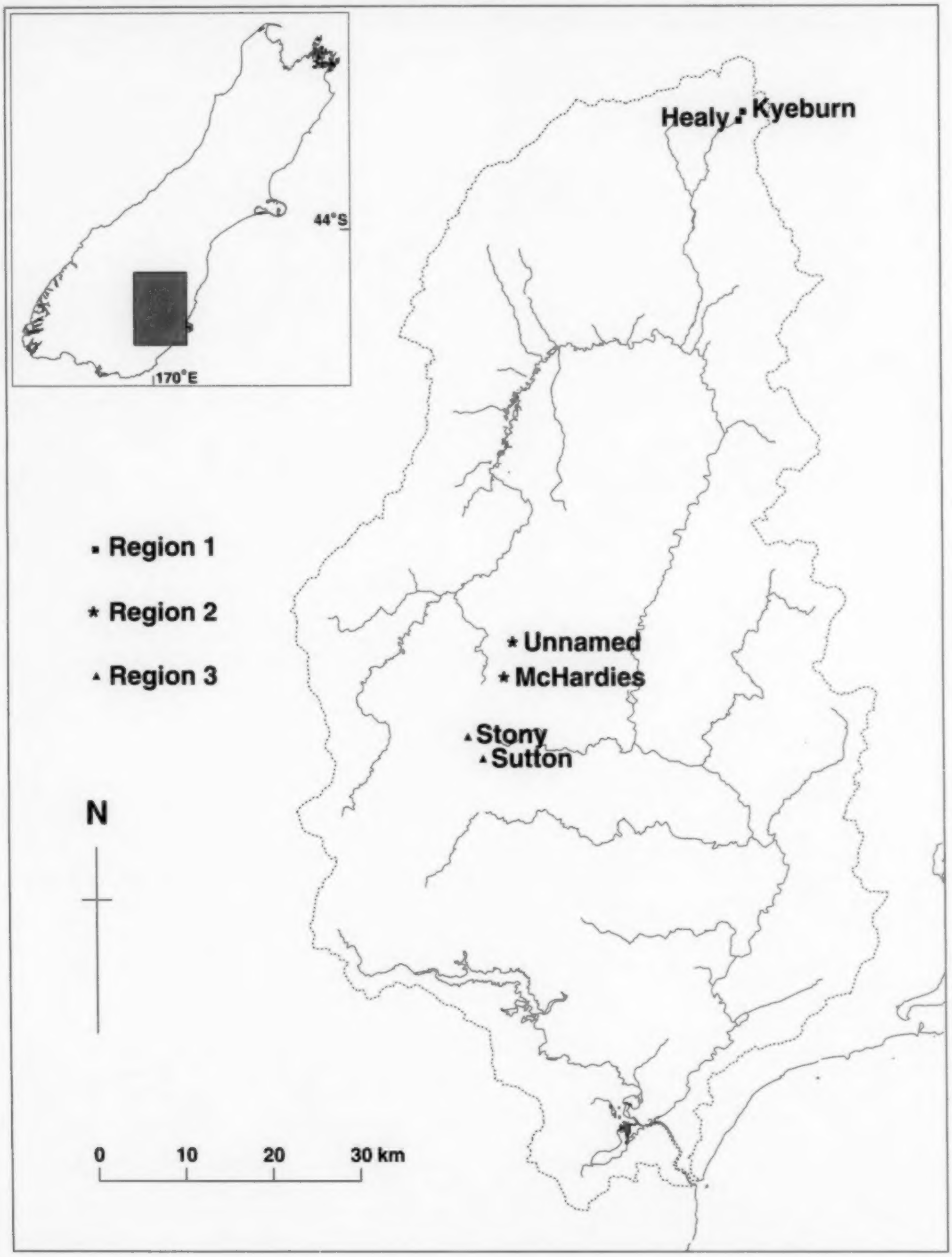
Periphyton communities on the streambed cobbles were dominated by red algae and diatoms. Cyanobacteria (*Nostoc* sp. and *Tolypothrix* sp.) were also abundant but only at two of the *Galaxias* sites.

The mean relative abundance of prostrate or unpalatable taxa was higher in the *Galaxias* streams than in the brown trout streams, and the mean relative abundance of erect taxa was generally higher in the trout streams (Table 1). However, while the fish type effect was significant (i.e., the effect of fish type was greater than the mean difference between transects within streams), the fish type effect was not significant for either variable when compared with interstream variation (Table 2). One pair of streams (Healy and Kyeburn) was distinctive in having no difference in the relative abundance of erect taxa.

Overall, streambed chlorophyll *a* was twofold higher and AFDM 1.4-fold higher in the trout streams than in the *Galaxias* streams (Table 1). However, as with the relative abundance of erect taxa, the differences did not occur in all the stream pairs, with Healy and Kyeburn (region 1) having similar biomass. The fish type effect was significant for both biomass variables in the overall ANOVA but was only significant for AFDM when the fish type effect was specifically tested using the interstream variance as an error term (Table 2). Percent chlorophyll *a* varied significantly as a function of fish type when compared with within-stream variance but not when compared with interstream variance (Table 2). While overall values were 1.4-fold higher in the trout streams than in the *Galaxias* streams, the results were inconsistent among the regions, with Healy (*Galaxias*) having a higher percent chlorophyll *a* than Kyeburn (trout) (Table 1).

There were no significant differences in P_{max}, CR, and the P_{max}:CR ratio as a function of fish type (Table 1), assessed

Fig. 1. Location map of study sites and regions in the Taieri River catchment, South Island, New Zealand.


44°S
170°E
Healy
Kyeburn
▪ Region 1
* Region 2
▴ Region 3
Unnamed
McHardies
Stony
Sutton
N
0
10
20
30 km

in terms of variability within or variability among streams (i.e., ANOVA, $p > 0.05$).

Overall, seston transport varied greatly as a function of fish type, with higher concentrations of both organic and inorganic seston in the *Galaxias* streams of two of the three regions (Table 1). On average, AFDM was twofold higher in the *Galaxias* streams than in the trout streams and ash was threefold higher. However, as with the previous variables, values for seston were very similar in Healy and Kyeburn. Thus, the overall differences were not significant when assessed as a function of interstream variability (Table 2).

Enrichment experiments

Chlorophyll *a* concentrations on the artificial substrata in

Table 2. ANOVAs of streambed periphyton variables and seston as a function of region and fish treatments.

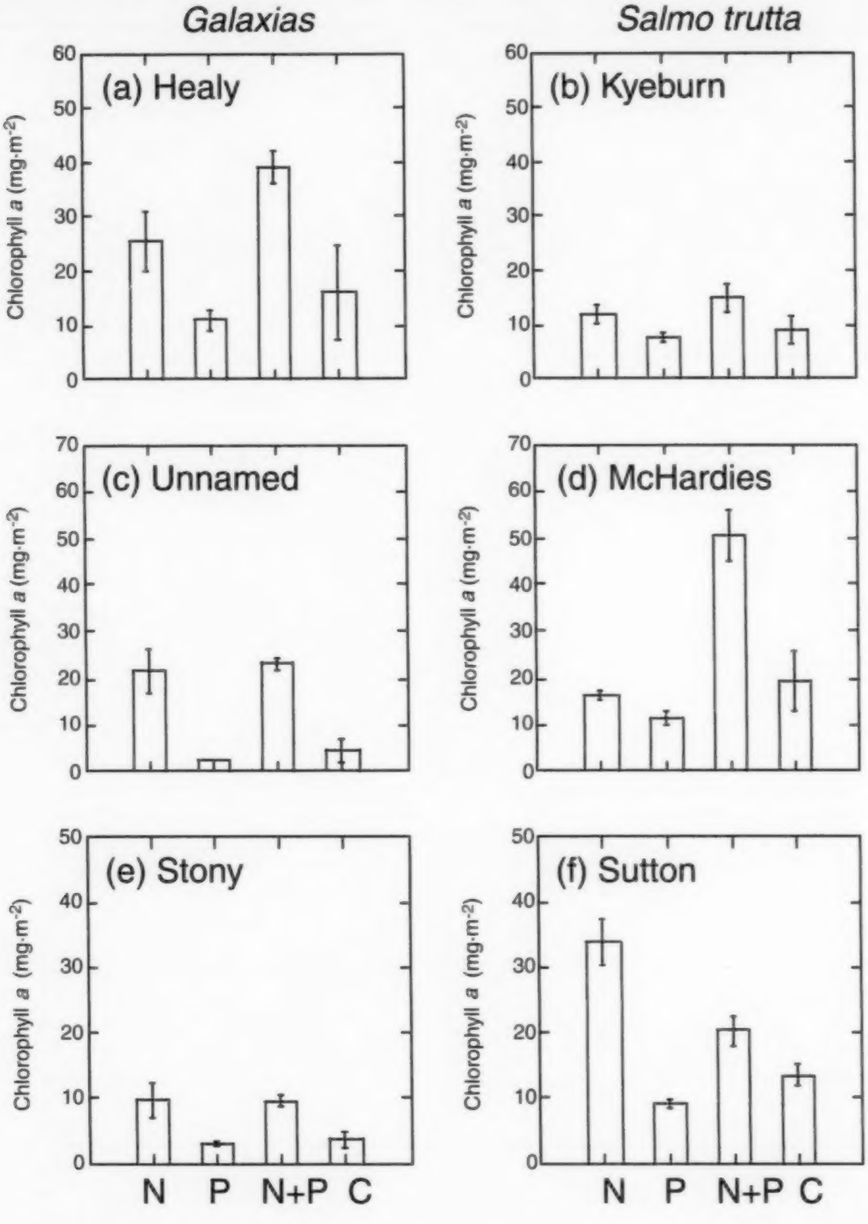
Source of variation	df	MS	*F*	*p*
Prostrate or unpalatable taxa				
Region	2	18.26	19.40	0.002
Fish	1	15.64	16.62	0.007
Region × fish	2	2.74	2.91	0.131
Error	6	0.94		
Hypothesis: effect = fish	1	15.64	5.72	0.139
Erect taxa				
Region	2	2.47	13.79	0.006
Fish	1	2.00	11.17	0.016
Region × fish	2	0.90	5.04	0.052
Error	6	0.179		
Hypothesis: effect = fish	1	2.00	2.21	0.275
Streambed chlorophyll *a*				
Region	2	73.85	34.75	0.001
Fish	1	94.98	44.70	0.001
Region × fish	2	33.10	15.58	0.004
Error	6	2.13		
Hypothesis: effect = fish	1	94.98	2.87	0.232
Ash-free dry mass				
Region	2	65.04	78.10	<0.001
Fish	1	11.85	14.24	0.009
Region × fish	1	0.57	0.69	0.538
Error	6	0.83		
Hypothesis: effect = fish	1	11.85	20.69	0.045
% chlorophyll *a*				
Region	2	0.01	20.68	0.002
Fish	1	0.01	15.48	0.008
Region × fish	1	0.01	23.53	0.001
Error	6	0.00		
Hypothesis: effect = fish	1	0.01	0.66	0.50
Organic seston				
Region	2	5.74	33.45	<0.001
Fish	1	8.68	50.62	<0.001
Region × fish	2	4.62	26.93	<0.001
Error	22	0.17		
Hypothesis: effect = fish	1	8.68	1.88	0.304
Inorganic seston				
Region	2	6.05	35.81	<0.001
Fish	1	5.97	35.32	<0.001
Region × fish	2	3.66	21.65	<0.001
Error	22	0.17		
Hypothesis: effect = fish	1	5.97	1.63	0.330

Note: The overall model is given together with a test of the effects of fish treatment using the $MS_{region×fish}$ interaction as the error term.

all streams were stimulated by N addition alone (indicating N limitation of periphyton growth) (Fig. 2; Table 3). Significant stimulation occurred in both *Galaxias* and trout streams and indicated that with enrichment, the production of the periphyton exceeded losses from grazers under both fish predation regimes, even though densities of the macrograzing caddisfly were higher in the *Galaxias* streams. However, the mean chlorophyll *a* level under enrichment was higher in the trout streams (28.56 mg·m^{-2} in trout streams versus 24.52 mg·m^{-2} in *Galaxias* streams), while the control levels were greatly different (10.93 versus 4.32 mg·m^{-2}, respectively; *t*-test, $p < 0.001$). Because of this large difference in background (control) biomass levels, there was a 5.7-fold increase in chlorophyll *a* with nutrient enrichment in the *Galaxias* streams compared with a 2.6-fold increase in chlorophyll *a* with enrichment in the trout streams (calculated as the ratio of N+P:control).

Some of the highly mobile mayfly nymphs escaped the sampling surfaces as soon as the samplers were approached in the stream, and snails were only present at two sites (at moderate to low densities). The attached or slow-moving invertebrate communities on the substrata were numerically dominated by chironomids that were embedded in the periphyton mats (cf. grazing on the surface). These insects

Fig. 2. Histograms (±1 SE) of periphyton chlorophyll *a* on N, P, N+P, and control (C) artificial substrata.



comprised >70% of the invertebrate densities on over half of the replicate substrata, so strongly influenced the results for differences in invertebrates among sites and fish treatments. The next most abundant group of invertebrates was caddisfly larvae, which comprised up to 30% of total invertebrate densities. The following analysis is therefore focused on total invertebrates, chironomids, and caddisflies.

Fish species alone did not have a significant effect on total invertebrate densities (Fig. 3; Table 4). However, there was a significant fish type effect on chironomid densities (Fig. 4). There were up to a 1000-fold higher densities of chironomids on the artificial substrates in the trout streams than in the *Galaxias* streams. The chironomids actively burrowed into the periphyton mat creating tube retreats. Indeed, there was a highly significant correlation between periphyton chlorophyll *a* and chironomid densities on the substrata ($r = 0.493$, $p < 0.001$, $n = 120$). Densities of caddisflies did not vary significantly as a function of fish treatment alone (Fig. 5; Table 4).

There was a highly significant difference in total invertebrates and caddisflies as a function of N enrichment (Table 4). This effect varied as a function of fish treatment, indicating that the manifestation of bottom-up N enrichment through to the second level of the food chain was dependent on the type of top-level fish predator present. For example, caddis larvae had 4.4-fold higher densities on enriched substrates (N+P:control) in the *Galaxias* streams versus only a 2.3-fold increase in the trout streams (Fig. 5). A similar difference occurred for the stimulation of total invertebrate densities (Fig. 3).

Discussion

Top-down trophic cascading is now widely recognized as an important ecological process, particularly in freshwater ecosystems (e.g., McQueen et al. 1989; Strong 1992; Forrester et al. 1999). Strong (1992) described such trophic cascades as meaning "runaway consumption, downward dominance through the food chain." In the typical top-down trophic cascade, overwhelming effects of predators propagate down through trophic levels, with the biomass and coverage of autotrophs being especially vulnerable. In the present study, we investigated how robust this paradigm is in natural, open-ended, trilevel trophic systems of streams with striking differences in the energetic demands and foraging behavior of the top-level predators. Could differences in predators, and indeed the introduction of brown trout into New Zealand streams, influence trophic cascading dynamics and the resultant ecosystem structure and function? We anticipated that during summer low flows, trout would exert a more significant top-down effect on autotrophy in our streams than the native *Galaxias* because of greater bioenergetic requirements and differences in predation strategies.

Autotrophs at the odd-linked level of the food chain are especially vulnerable to changes in grazing pressure that might accompany shifts in predation intensity (Power 1992; Strong 1992). Indeed, the physiognomy of periphyton is considered a useful indicator of variations in herbivore activity (Steinman 1996; McCollum et al. 1998). Algal taxa with erect or filamentous growth forms are more prone to herbivory than prostrate taxa (Steinman 1996). Also, grazing appears to promote the relative abundance of some cyanobacteria that are probably unpalatable (or at least are selected against) (Rosemond et al. 1993). The results of this study generally supported our hypothesis of reduced herbivory in the trout streams relative to the *Galaxias* streams but lacked statistical significance. In particular, the erect, complexly branched filamentous red alga *Audouinella hermanii* was dominant in two of the three trout streams, and cynanobacterial taxa (*Nostoc* sp., and *Tolypothrix* sp.) were common, and only recorded, in two of the three *Galaxias* streams. Of note was that while the trout streams were dominated by the more erect overstory taxa, most of these were also present in the *Galaxias* streams but at a much lower abundance (indicating that they were usually not excluded by the higher grazing pressure in the *Galaxias* streams). These taxa were apparently permitted to outgrow the prostrate understory taxa in the trout streams. Thus, there was a subtle shift in the relative abundance of some taxa as a function of top-down control rather than a gross shift in community composition.

Table 3. Split-plot ANOVA of chlorophyll *a* on artificial substrates as a function of region, fish, N, and P treatments.

Source of variation	df	MS	*F*	*p*
Region	2	0.17	0.13	0.886
Fish	1	1.19	0.88	0.447
Residual (region × fish)	2	1.35	13.58	
N	1	6.23	62.55	<0.001
P	1	0.10	1.03	0.330
Fish × N	1	0.54	5.42	0.038
Fish × P	1	0.01	0.15	0.710
N × P	1	0.067	0.67	0.428
Fish × N × P	1	0.04	0.36	0.559
Residual (region × fish × N × P)	12	0.10	9.68	
Error	96	0.01		
Total	119			

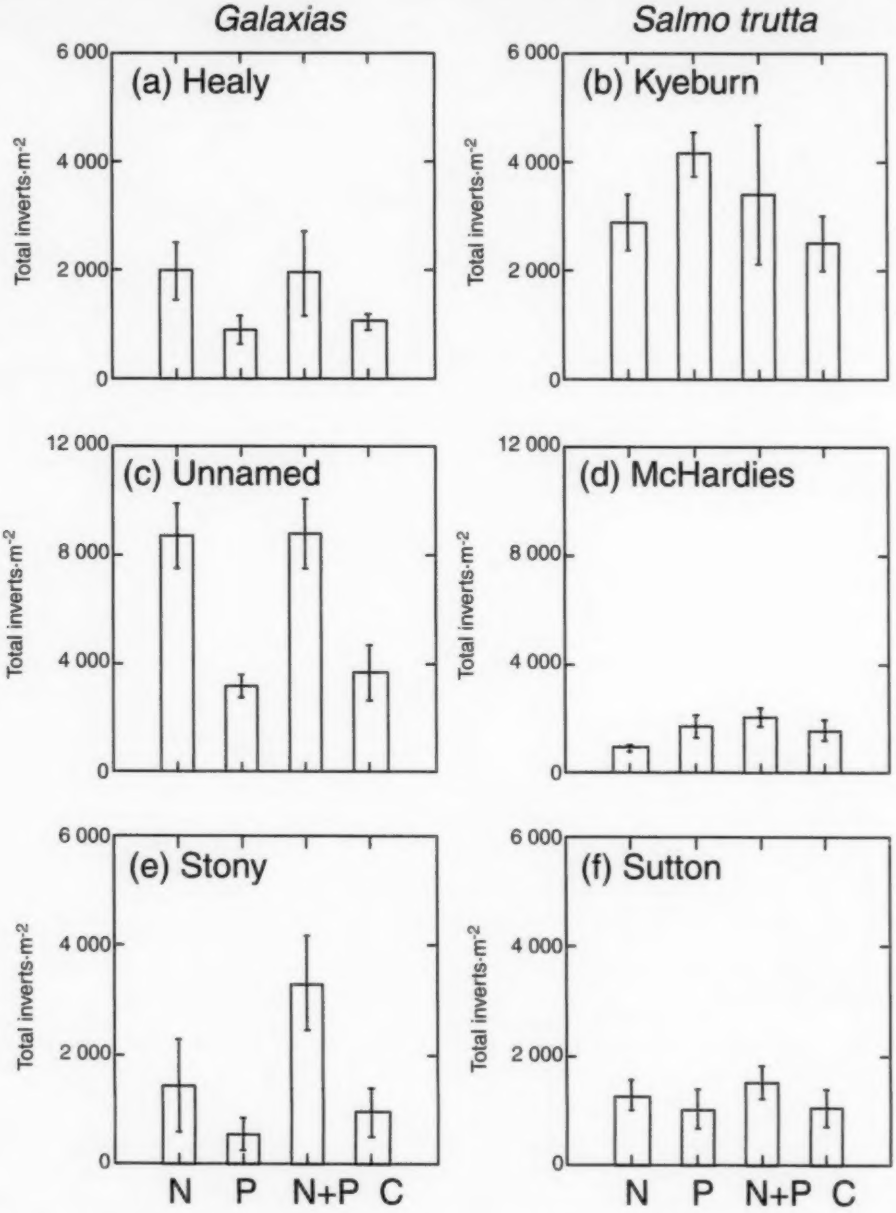
Note: The effects of fish and N and P treatments were tested using the residual error term as shown in the table.

These differences in periphyton community architecture translated into differences in biomass on the streambed as a function of fish predation regime. The differences in chlorophyll *a* and AFDM between fish treatments were dramatic for two of the three pairs of streams and for these streams supported the hypothesis of greater top-down control of autotrophy in the *Galaxias* streams. Two main mechanisms might contribute to the observed differences in top-down trophic cascade effects between the two predators. First, production budget analysis for two of our study streams by Huryn (1998) has shown that trout production in Sutton Stream is almost an order of magnitude greater than *Galaxias* production in Stony Creek. Also, only 18% of annual benthic invertebrate production was required to support *Galaxias* growth in Stony Creek, whereas essentially all available prey production was required to support trout production in Sutton Stream. Second, trout, being daytime visual feeders, probably exert a stronger predation pressure than the nighttime *Galaxias* feeders by virtue of the 16 h of daylight versus 8 h of dark that occurs during summer in these southern (>45°S) streams (McIntosh and Townsend 1995*a*, 1996).

Region 1 streams (Healy and Kyeburn) did not fully agree with our hypotheses or conform with results from region 2 and 3 streams for streambed periphyton communities. The reason for this might be that, unlike the other pairs of streams, Healy and Kyeburn had similar biomass of fish (and by inference, bioenergetic requirements). Indeed, trout biomass was low in Kyeburn relative to the other trout streams. Thus, the fish in these streams are likely to exert similar predation pressure on the benthic consumers because a similar level of fish biomass is being supported. This infers that the mechanisms generating the differences in periphyton and seston between *Galaxias* and trout streams in regions 2 and 3 were probably related more to differences in bioenergetic requirements than to predation strategies of the fish.

To verify more conclusively the relative top-down versus bottom-up control in the *Galaxias* versus trout streams, we also attempted to stimulate periphyton biomass with nutrient enrichment. We hypothesized that chlorophyll *a* responses to enrichment should be greater in the trout streams than in the *Galaxias* streams because of greatly reduced top-down con-

Fig. 3. Histograms (±1 SE) of total invertebrate densities on N, P, N+P, and control (C) artificial substrata.



trol of periphyton in the trout streams. However, we found that enrichment resulted in significant increases in chlorophyll *a* under both fish treatments. This occurred even though there were much higher densities of invertebrates with enrichment in the *Galaxias* streams that should have prevented the enrichment manifesting as an increase in periphyton biomass, as found in a neighboring catchment (Biggs and Lowe 1994). It appears that the increased consumption with higher densities of herbivores in the *Galaxias* streams was still insufficient to control the enrichment effects on periphyton. Thus, while periphyton biomass was predominantly controlled by top-down forces under ambient resource supplies in the *Galaxias* streams, production appeared to be very sensitive to changes in bottom-up resource supply as well. Rosemond et al. (1993) reported that periphyton chlorophyll *a* in a Tennessee woodland stream with low nutrient supplies was affected to a greater extent by nutrients than by grazers. We suggest that this was probably also the case in our study streams, at least over the short time frame of our experiments, which was insufficient for the increased primary production to be manifested as increased invertebrate fecundity and then densities (assuming

Table 4. Split-plot ANOVAs of mean invertebrate densities on artificial substrates as a function of region, fish, N, and P treatments.

Source of variation	df	MS	*F*	*p*
Total invertebrate density				
Region	2	5.46×10^7	0.56	0.642
Fish	1	2.27×10^7	0.23	0.678
Residual (region × fish)	2	9.83×10^7		
N	1	6.15×10^7	9.74	0.009
P	1	6.83×10^6	1.08	0.319
Fish × N	1	5.48×10^7	8.68	0.012
Fish × P	1	1.272×10^6	0.20	0.661
N × P	1	5.52×10^5	0.09	0.772
Fish × N × P	1	1.70×10^5	0.03	0.872
Residual (region × fish × N × P)	12	6.31×10^6	5.23	
Error	96	1.21×10^6		
Total	119	6.45×10^8		
Chironomid density				
Region	2	2.34×10^7	25.14	0.038
Fish	1	5.28×10^7	56.80	0.017
Residual (region × fish)	2	9.29×10^5		
N	1	6.81×10^5	0.96	0.348
P	1	9.81×10^5	1.38	0.264
Fish × N	1	6.81×10^5	0.96	0.348
Fish × P	1	3.50×10^6	4.91	0.047
N × P	1	5.12×10^5	0.72	0.414
Fish × N × P	1	2.73×10^4	0.04	0.848
Residual (region × fish × N × P)	12	7.13×10^5	1.75	
Error	96	3.92	4.08	
Total	119	1.55×10^8		
log caddisfly density				
Region	2	108.73	0.81	0.553
Fish	1	25.58	0.19	0.706
Residual (region × fish)	2	134.70	43.50	
N	1	28.63	9.25	0.010
P	1	6.49	2.10	0.173
Fish × N	1	25.16	8.13	0.015
Fish × P	1	1.45	0.47	0.507
N × P	1	24.27	7.84	0.016
Fish × N × P	1	3.44	1.11	0.313
Residual (region × fish × N × P)	12	3.10	0.48	
Region × stream × nutrients	96	614.40	6.40	
Total	119	1253.44		

Note: The effects of fish, N, and P treatments were tested using the residual error term as shown in the table.

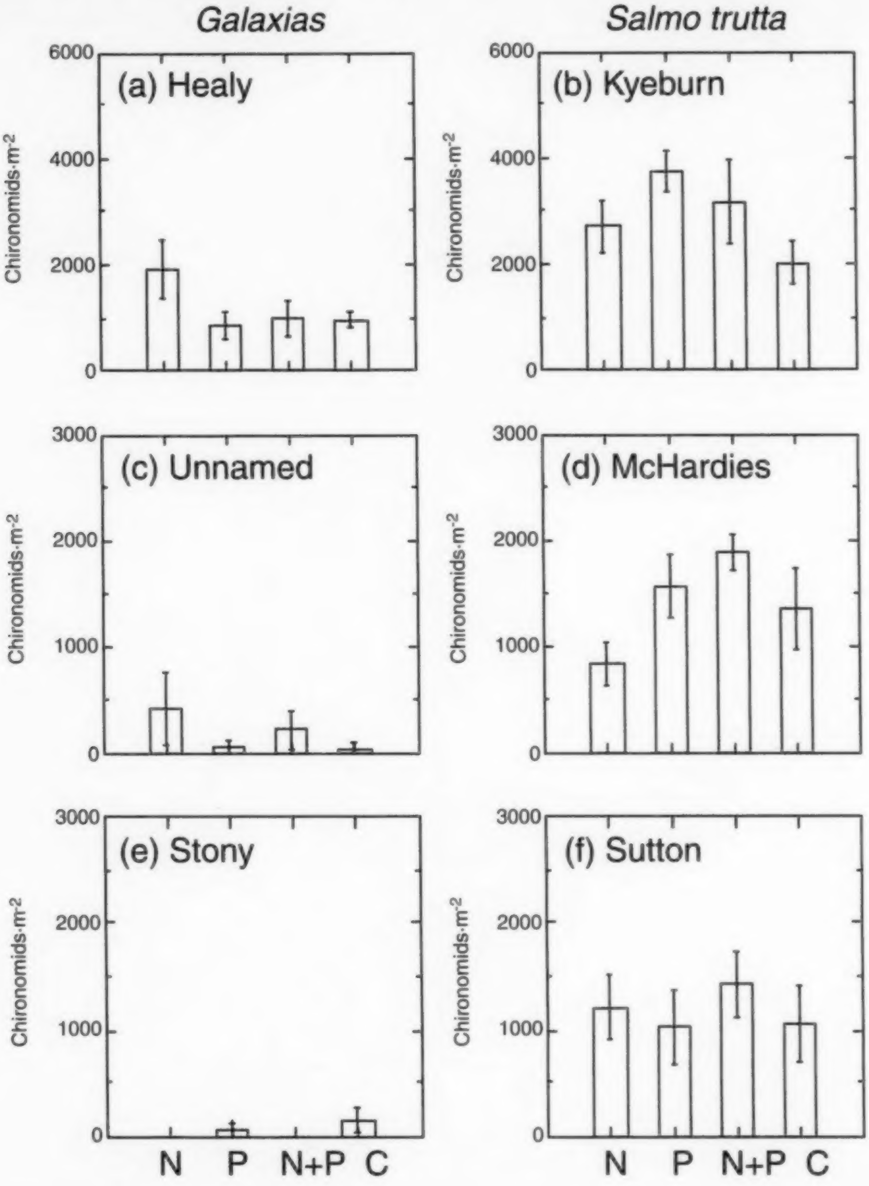
that food resources were limiting). Conversely, while predation control of invertebrates was apparently significantly lower in the *Galaxias* streams than in the trout streams, it may still have been sufficient to prevent a more significant response of the invertebrates to increased primary production.

Results from the benthic metabolism experiments did not provide any support for our hypotheses. Effects of different herbivory regimes on periphyton metabolism have not been found in other studies. Both the $P_{\max}$ and the CR results were highly variable and did not appear to be very sensitive to the differences in benthic chlorophyll *a* and percent chlorophyll *a* that were recorded among the streams. Chlorophyll *a* concentration is often a significant correlate of benthic $P_{\max}$ in streams (e.g., Dodds et al. 1999).

Transported seston indicates the effects of differences in invertebrate numbers and activity through changes in invertebrate feces production, organic matter processing, and dislodgment of periphyton. Removal of invertebrates can cause substantial reductions in seston transport (e.g., Wallace et al. 1991). Results from two of the three pairs of streams supported the hypothesis that trout are reducing invertebrate density (McIntosh and Townsend 1996) and causing invertebrates to limit their activity during the day (McIntosh and Townsend 1994, 1995*b*). Seston is an important food source for many members of river communities (Wallace and Merrit 1980; Benke et al. 1988), so reductions in supply of seston in trout streams may cause bottom-up limitation of filtering invertebrate populations. However, the increase in periphyton biomass in trout streams may counter this effect, since there are likely to be high rates of sloughing during spates (e.g., Biggs and Close 1989).

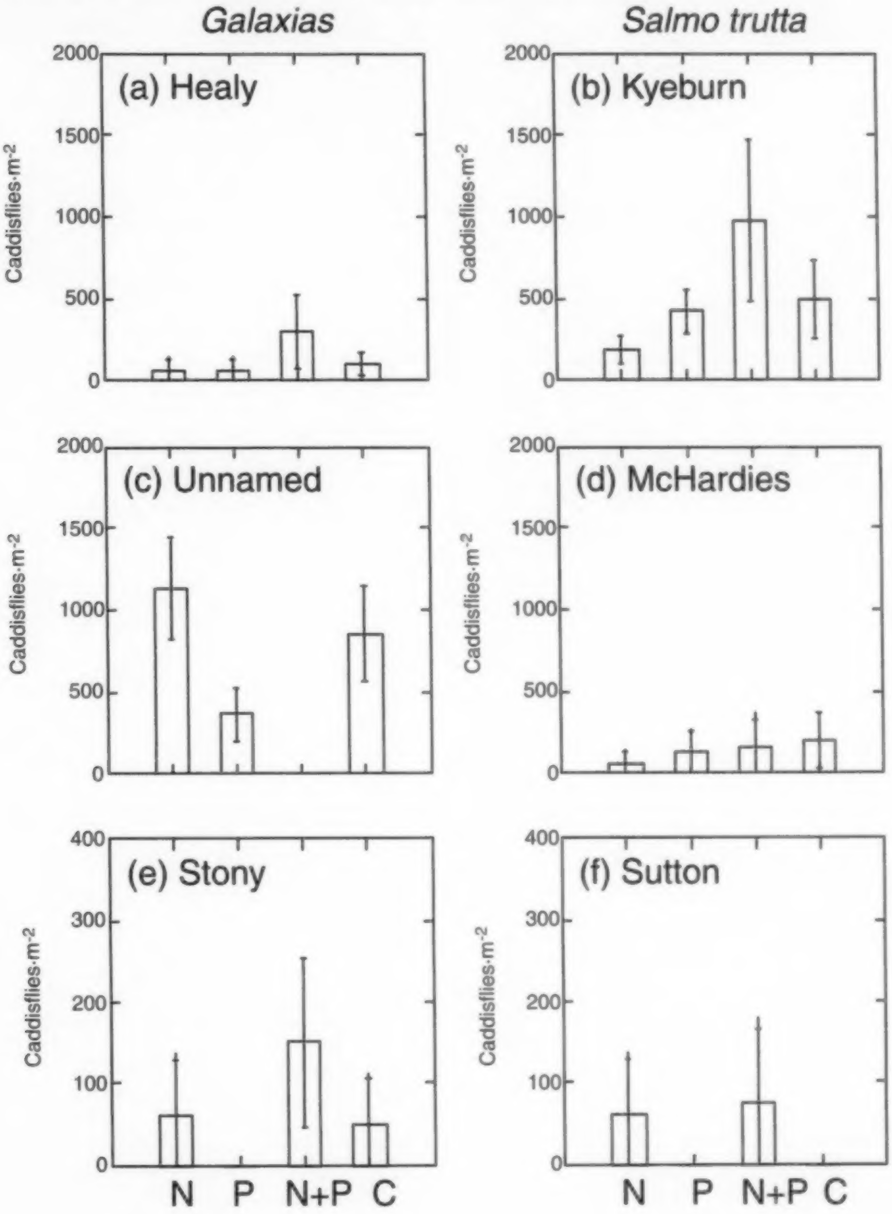
We expected that with lower predation pressure on grazers

Fig. 4. Histograms (±1 SE) of chironomid densities on N, P, N+P, and control (C) artificial substrata.



in the *Galaxias* streams, there should be a bottom-up trophic cascade if periphyton are enriched, leading to a stimulation of invertebrate densities. However, we did not expect this to occur in the trout streams because of stronger predation control of the herbivores. This was based on the assumption that periphyton production in these grassland streams was nutrient limited, as has been shown previously for streams in the neighboring Kakanui River catchment (Biggs and Lowe 1994) and in the Kyeburn (Francoeur et al. 1999). It was also assumed that the mobile or drifting grazers would be resource limited and that they would track food production through a "random walk" type scenario and congregate on patches of enhanced food production if predation pressure allowed. Indeed, Hart (1981) found in experiments without predation pressure that a foraging caddis larva (*Dicosmoecus gilvipes*) spent up to 97% of its time feeding and that it moved very little if there was sufficient food in the foraging area. Our manipulation of nutrients did result in a significant stimulation of periphyton chlorophyll *a* in all streams, which suggests that periphyton growth rates at all sites were nutrient limited regardless of fish predation regime. This stimulation of periphyton production also translated into a significant stimulation of total numbers of invertebrates and caddis larvae on the artificial substrata, and this stimulation

Fig. 5. Histograms (±1 SE) of caddisfly densities on N, P, N+P, and control (C) artificial substrata.



was much greater in the *Galaxias* streams than in the trout streams. Invertebrates were relatively less constrained by predation threat from migrating onto the open substratum in the *Galaxias* streams and so were able to focus their grazing activity where primary production was highest.

The response of invertebrates to enrichment of the periphyton suggests that the herbivores were food limited at background rates of periphyton production. Indeed, based on a production budget from Stony Creek, Huryn (1998) found that herbivores consumed virtually all primary production in the streams (i.e., surplus not significantly different from zero), which indicates extremely close coupling between herbivore and primary production and the high likelihood of food limitation in Stony Creek. Conversely, there was no significant response (or even weak trends) in invertebrate numbers with nutrient enrichment in the trout streams, suggesting a predominance of top-down control in these streams. However, because caddis densities were much lower on the substrata in these streams, we also cannot reject the possibility that satiated growth was occurring with existing rates of periphyton production so that a concentration of feeding activity on "hotspots" of high periphyton production was not necessary. We believe it more likely that foraging excursions of the macrograzers were being restricted as a behavioral response to increased trout predation pressure, as shown for a number of other stream invertebrates (e.g., McIntosh and Townsend 1994).

The positive correlation between chironomid density and periphyton chlorophyll *a* suggests that the chironomids were cueing into increased habitat availability/refugia, but perhaps without having a significant effect on periphyton biomass in the trout streams (because the correlation coefficient was positive). Most of these chironomids were embedded in the periphyton mat, and so it is unlikely that they would have been vulnerable to predation by either trout or *Galaxias*. Strong correlations between periphyton biomass and chironomid densities have been found in several other southern New Zealand rivers, which are renowned as brown trout fisheries (B.J.F. Biggs, unpublished data). Forrester et al. (1999) found that the presence of dace in experimental channels also increased chironomid densities relative to controls because of increased periphyton biomass. Huryn (1998) reported the same link between chironomid densities and periphyton cover as a function of trout versus *Galaxias* predation strategies in his study of Sutton Stream and Stony Creek. Thus, heterogeneity in predation regimes was mediating the occurrence of a two trophic level, bottom-up response in the trout stream food webs while simultaneously causing top-down control of the large herbivores inhabiting the upper canopy of the periphyton mat. While such dynamic feedbacks have been recently discussed in the conceptual model of Hunter and Price (1992), they have yet to be incorporated into mathematical models of food chain dynamics (Power 1992).

Two important groups of invertebrates that could not be enumerated successfully in this study because of their high mobility were the mayfly and stonefly herbivores. The mayflies are probably important in generating the periphyton responses because (*i*) they can be efficient at reducing periphyton biomass on stone surfaces (through processes of consumption and physical dislodgment; Scrimgeour et al. 1991) and (*ii*) they are very mobile and respond rapidly to the presence of predators (e.g., McIntosh and Townsend 1994) by vacating exposed areas. Indeed, it is this latter facility of the mayflies that prevented us from precisely enumerating them. As soon as the substrata were approached for collection, mayflies were observed leaving the substrate sampler surfaces.

Implications for trophic cascading theory

The now classical theory of Oksanen et al. (1981) predicts a stepped pattern of biomass accrual across gradients in productivity. To summarize, in systems with an odd number of trophic levels (such as the trilevel streams in the present study), increases in primary production through enhancement of growth-limiting resources should result in increased biomass of the odd-numbered trophic levels and no change in biomass for the even-numbered trophic levels. However, in systems with an even number of trophic levels, enhancement of primary production should result in increased biomass for even-numbered trophic levels and no change in biomass in odd-numbered trophic levels. In our study streams, nutrient enrichment resulted in an enhancement of primary producer biomass in both *Galaxias* and trout streams, as predicted by Oksanen et al's. (1981) models for trilevel systems. However, this enhancement was not equal among the fish predation treatments. Of particular significance was that nutrient enhancement of autotrophic production also resulted in a stimulation of the even-numbered trophic level (total invertebrate grazers, particularly the caddisflies), with this stimulation being significantly higher in the *Galaxias* streams. This result is predicted for an even number of trophic systems, not the three-level systems of our study streams.

Two major conclusions can be drawn from our open-ended stream results in relation to trophic cascading theory. First, bottom-up forces are of fundamental significance in these trilevel food chains, regardless of heterogeneity in predator type/strategy. Thus, as noted recently by Stiling and Rossi (1997) in a terrestrial cascading study, "bottom-up forces set the stage on which top-down forces act." A similar result has been reported by Mazumder (1994) and Brett and Goldman (1997) for freshwater pelagic food chains. Second, the response of the even-numbered level in the food chain to enrichment in a trilevel system is very sensitive to the type of predator dominating the top trophic level. In other words, the response of the even-numbered trophic level in a trilevel system to bottom-up enhancement of primary producer growth is sensitive to heterogeneity in predation regime. It suggests that bottom-up control is stronger at the base of the food chain and that top-down control is stronger at higher trophic levels, as proposed by McQueen et al. (1986). Our results also infer that the identities of individual species are as important in trophic cascading effects as the number of levels in a food chain (also see Hunter and Price 1992).

The wealth of data demonstrating top-down and bottom-up influences on stream periphyton communities clearly suggests that the number of trophic levels is potentially an important factor structuring stream ecosystems during periods of physical stability. However, there are still few data from real streams to judge the applicability of theoretical models of trophic cascading, and more studies need to address the effects of different predation strategies among top-level animals. Our results suggest that *Galaxias* fish may not be operating as a "functionally significant" top-level predator in New Zealand streams (at least not relative to trout) and that streams dominated by *Galaxias* are less likely to show autotrophic responses to long-term nutrient enrichment (e.g., through land-use change) than streams containing brown trout. Because of this heterogeneity in predation strategies, the *Galaxias* streams appear to be more closely akin to systems with two trophic levels than to those with three levels. This has important implications for the way that we characterize the number of functional trophic levels and top-down versus bottom-up ecosystem control based on information such as feeding guilds. In terms of conservation biology, it also adds to our understanding of the potential effects of aquatic species introductions into geographically isolated island environments. At the end of the Introduction, we posed the question whether trout streams are greener than *Galaxias* streams. Our results suggest that during hydrologically stable periods, the answer probably is yes. Thus, it is clear that trophic cascading in the trout streams supports the hypothesis of Hairston et al. (1960) that "the world is green" because predators keep herbivores in check. However, our results from the *Galaxias* streams are quite contrary to this theory because the predators do not significantly control the herbivores. Overall, our study provides strongest support for the suggestion by Hunter and Price (1992) and Power (1992) that heterogeneity in biotic factors such as the top-level predators can strongly effect the nature and strength of trophic cascades in natural ecosystems.

Acknowledgments

We are grateful for the assistance of Rob Smith, Lachlan Kirk, and Carleen Mitchell with field work and laboratory analyses, David Baird (Biometrician, AgResearch Ltd.) for statistical advice, and Liz Bergey, Mary Power, and Jim Bowlby for their constructive reviews of this manuscript. This work was supported by FRST Programs "Environmental Hydrology and Habitat Hydraulics" (contract CO1519 to NIWA) and "Taieri and Southern Rivers" (contract to Otago University).

References

Benke, A.C., Hall, C.A.S, Hawkins, C.P., Lowe-McConnell, R.H., Stanford, J.A., Suberkropp, K., and Ward, J.M. 1988. Bioenergetic considerations in the analysis of stream ecosystems. J. North Am. Benthol. Soc. **7**: 480–502.

Biggs, B.J.F., and Close, M.E. 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biol. **22**: 209–231.

Biggs, B.J.F., and Lowe, R.L. 1994. Responses of two trophic levels to patch enrichment along a New Zealand stream continuum. N.Z. J. Mar. Freshwater Res. **28**: 119–134.

Brett, M.T., and Goldman, C.R. 1997. Consumer versus resource control in freshwater pelagic food webs. Science (Washington, D.C.), **275**: 384–386.

Bronmark, C., Klosiewski, S.P., and Stein, R.A. 1992. Indirect effects of predation in a freshwater, benthic food chain. Ecology, **73**: 1662–1674.

Crowl, T.A., Townsend, C.R., and McIntosh, A.R. 1992. The impact of introduced brown and rainbow trout on native fish: the case of Australasia. Rev. Fish Biol. Fish. **2**: 217–241.

Dodds, W.K., Biggs, B.J.F., and Lowe, R.L. 1999. Photosynthesis–irradiance patterns in benthic microalgae: variations as a function of assemblage thickness and community structure. J. Phycol. **35**: 42–53.

Elner, R.W., and Vadas, R.L. 1990. Inference in ecology: the sea urchin phenomenon in the northwestern Atlantic. Am. Nat. **136**: 108–125.

Flecker, A.S., and Townsend, C.R. 1994. Community-wide consequences of trout introduction in New Zealand streams. Ecol. Appl. **4**: 798–807.

Forrester, G.E., Dudley, T.L., and Grimm, N.B. 1999. Trophic interactions in open systems: effects of predators on stream food chains. Limnol. Oceanogr. **44**: 1187–1197.

Francoeur, S.N., Biggs, B.J.F., Smith, R.A., and Lowe, R.L. 1999. Nutrient limitation of algal biomass accrual: seasonal patterns and comparison of methodology. J. North Am. Benthol. Soc. **18**: 242–260.

Fretwell, S.D. 1977. The regulation of plant communities by food chains exploiting them. Perspect. Biol. Med. **20**: 169–185.

Hairston, N.G., Smith, F.E., and Slobodkin, L.B. 1960. Community structure, population control, and competition. Am. Nat. **94**: 421–424.

Hart, D.D. 1981. Foraging and resource patchiness: field experiments with a grazing stream insect. Oikos, **37**: 46–52.

Hunter, M.D., and Price, P.W. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology, **73**: 724–732.

Huryn, A.D. 1998. Ecosystem-level evidence for top-down and bottom-up control of production in a grassland stream system. Oecologia, **115**: 173–183.

Mazumder, A. 1994. Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. Ecology, **75**: 1141–1149.

McCollum, E.W., Crowder, L.B., and McCollum, S.A. 1998. Complex interactions of fish, snails, and littoral zone periphyton. Ecology, **79**: 1980–1994.

McIntosh, A.R., and Townsend, C.R. 1994. Interpopulation variation in mayfly anti-predator tactics: differential effects of contrasting predatory fish. Ecology, **75**: 2078–2090.

McIntosh, A.R., and Townsend, C.R. 1995*a*. Contrasting predation risks presented by introduced brown trout and native common river *Galaxias* in New Zealand streams. Can. J. Fish. Aquat. Sci. **52**: 1821–1833.

McIntosh, A.R., and Townsend, C.R. 1995*b*. Impacts of an introduced predatory fish on mayfly grazing in New Zealand streams. Limnol. Oceanogr. **40**: 1508–1512.

McIntosh, A.R., and Townsend, C.R. 1996. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behaviour? Oecologia, **108**: 174–181.

McQueen, D.J., Post, J.R., and Mills, E.L. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. **43**: 1571–1581.

McQueen, D.J., Johannes, J.R., and Mills, E.L. 1989. Bottom-up and top-down impacts on freshwater pelagic community structure. Ecol. Monogr. **59**: 289–309.

Nisbet, R.M., Diehl, S., Wilson, W.G., Cooper, S.D., Donalson, D.D., and Kratz, K. 1997. Primary-productivity gradients and short-term population dynamics in open systems. Ecol. Monogr. **676**: 535–553.

Oksanen, L., Fretwell, S.D., Arruda, J., and Neimela, P. 1981. Exploitation ecosytems in gradients of primary productivity. Am. Nat. **118**: 240–261.

Power, M.E. 1992. Top down and bottom up forces in food webs: do plants have primacy. Ecology, **73**: 733–746.

Rosemond, A.D., Mullholland, P.J., and Elwood, J.W. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology, **74**: 1264–1280.

Sala, E., Boudouresque, C.F., and Harmelin-Vivien, M. 1998. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. Oikos, **82**: 425–439.

Scrimgeour, G.J., Culp, J.M., Bothwell, M.L., Wrona, F.J., and McKee, M.H. 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly–diatom systems. Oecologia, **85**: 343–348.

Steinman, A.D. 1996. Effects of grazers on freshwater benthic algae. *In* Algal ecology: freshwater benthic ecosystems. *Edited by* R.J. Stevenson, M.L. Bothwell, and R.L. Lowe. Academic Press, San Diego, Calif. pp. 341–373.

Stiling, P., and Rossi, A.M. 1997. Experimental manipulations of top-down and bottom-up factors in a tri-trophic system. Ecology, **78**: 1602–1606.

Strong, D.R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. Ecology, **73**: 747–754.

Townsend, C.R., Thompson, R.M., McIntosh, A.R., Kilroy, C., Edwards, E., and Scarsbrook, M.R. 1998. Disturbance, resource supply and food web architecture in streams. Ecol. Lett. **1**: 200–209.

Wallace, J.B., and Merritt, R.W. 1980. Filter-feeding ecology of aquatic insects. Annu. Rev. Entomol. **25**: 103–132.

Wallace, J.B., Cuffney, T.F., Webster, J.R., Lugthart, G.J., Chung, K., and Godowitz, B.S. 1991. Export of fine organic particles from headwater streams: effects of season, extreme discharges, and invertebrate manipulation. Limnol. Oceanogr. **36**: 670–682.

A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web

Chris J. Harvey and James F. Kitchell

Abstract: We used stable isotope analysis to derive trophic relationships and movement patterns for components of the western Lake Superior food web. Trophic linkages implied by previous gut content studies were only marginally supported by stable isotope data. Siscowet lake trout (*Salvelinus namaycush siscowet*) were the top predators, and trophic overlap between siscowet and lean lake trout (*Salvelinus namaycush*) was low. Exotic Pacific salmon (*Oncorhynchus* spp.) occupied a lower trophic position than native piscivores because the latter relied more on coregonids. To evaluate spatial heterogeneity of the food web, we assumed that the adjacent cities of Duluth and Superior (DS) were a point source of ^{15}N, and we measured isotopes of organisms close to and far from DS. Slimy sculpin (*Cottus cognatus*) were enriched in the DS area relative to other sites, implying that they are relatively sedentary. Rainbow smelt (*Osmerus mordax*) showed no differences at any sites, implying high vagility. Other organisms showed differences that could not be attributed to DS, implying that other mechanisms, such as trophic ontogeny, were influencing their isotopic signatures.

Résumé : Nous avons eu recours à l'analyse des isotopes stables pour établir les relations trophiques et les patrons de déplacement des composantes du réseau trophique dans l'ouest du lac Supérieur. Les liens trophiques supposés à la suite d'études antérieures sur le contenu de l'intestin n'ont été que faiblement étayés par les données sur les isotopes. Le principal prédateur était le touladi gras (siscowet) (*Salvelinus namaycush siscowet*), et on observait un faible chevauchement trophique entre cette sous-espèce et le touladi maigre (*S. namaycush*). Les espèces exotiques de saumons du Pacifique (*Oncorhynchus* spp.) occupaient une position trophique inférieure à celle des piscivores indigènes, ces derniers s'appuyant davantage sur les corégonidés. Pour évaluer l'hétérogénéité spatiale du réseau trophique, nous avons posé que les cités voisines de Duluth et Superior (DS) constituaient une source ponctuelle de ^{15}N, et nous avons dosé les isotopes chez des organismes présents à proximité et loin de DS. Chez le chabot visqueux (*Cottus cognatus*), la teneur était plus forte dans la région de DS qu'à tous les autres sites, ce qui signifierait que ce poisson est relativement sédentaire. Chez l'éperlan arc-en-ciel (*Osmerus mordax*), on n'a noté aucune différence d'un site à l'autre, ce qui indique une grande mobilité. D'autres organismes présentaient des différences qui ne pouvaient pas être attribuées à DS, ce qui signifie que d'autres mécanismes, comme l'ontogénèse trophique, influaient sur les signatures isotopiques qu'ils portaient.

[Traduit par la Rédaction]

Introduction

Stable isotope analysis has been used extensively to describe aquatic food webs. The ratio of the stable isotopes of nitrogen (^{15}N:^{14}N) is positively correlated with trophic level, and the ratio of carbon stable isotopes (^{13}C:^{12}C) yields information about the production base of the food web (e.g., Peterson and Fry 1987). Stable isotope analysis offers a complement to gut content analysis; gut contents provide a detailed look at an organism's most recent feeding habits, and stable isotopes integrate a longer temporal scale of feeding, assimilation, and growth (Kling et al. 1992; Yoshioka et al. 1994). Recent studies demonstrate other uses of stable isotope analysis in aquatic ecology. For example, Hansson et al. (1997) used a ^{15}N point source, a sewage treatment plant, to elicit movement patterns of fishes in the Baltic Sea. Several studies (e.g., Cabana and Rasmussen 1994; Kidd et al. 1995; Kiriluk et al. 1995) have shown positive relationships between ^{15}N levels and biocontaminant concentrations in aquatic food webs, and ^{15}N levels in aquatic taxa are positively correlated with human populations in their catchment (Cabana and Rasmussen 1996). Thus, stable isotope patterns may reflect food web characteristics, life history traits and movement patterns of aquatic taxa, and human impacts in aquatic ecosystems.

Information on trophic positions, life histories, and movement patterns of key species is lacking for Lake Superior, the largest of the Laurentian Great Lakes. Lake Superior is the only one of the Great Lakes that has a relatively intact native fish community. In the other lakes, lake trout (*Salvelinus namaycush*) were extirpated by overfishing and sea lamprey (*Petromyzon marinus*) predation in the mid-1900s, and the native coregonids (*Coregonus* spp.) have largely been replaced by exotics like the alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) (Christie 1974). Lake Superior maintains naturally reproducing lake trout and large populations of native coregonids,

Received July 13, 1999. Accepted March 22, 2000.
J15249

C.J. Harvey[1] and J.F. Kitchell. Center for Limnology, University of Wisconsin-Madison, Madison, WI 53706, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: cjharvey@students.wisc.edu

although exotic rainbow smelt and Pacific salmon (*Oncorhynchus* spp.) populations have become established.

A key issue in Lake Superior is the predatory demand of salmonids on forage fish populations. Recent modeling studies concluded that the forage fish biomass in western Lake Superior was insufficient to meet the demands of predators and commercial and tribal harvests (Ebener 1995; Negus 1995). Fisheries managers, who desire to restore lake trout populations to pre-1940s levels (Busiahn 1990), must determine the importance of various prey species and the degree of trophic overlap between native and exotic salmonids to better resolve the predator–prey imbalance, allocate fish biomass, and meet lake trout restoration goals. Stable isotope analysis of the offshore food web will help provide such information to managers. Keough et al. (1996) used stable isotope analysis to examine the food webs of a Lake Superior wetland and nearshore community, but food web studies of the offshore community have been limited to gut content analyses (e.g., Anderson and Smith 1971; Selgeby 1988; Conner et al. 1993).

A large human population center, the adjacent ports of Duluth and Superior at the southwest corner of Lake Superior (Fig. 1), is a potential point source of ^{15}N that may be useful for studies of the offshore community. The population density of the Duluth–Superior (DS) area is two orders of magnitude greater than surrounding areas, and surface water concentrations of ammonium near Duluth are at least an order of magnitude greater than the lakewide average (Weiler 1978; Rathke and McRae 1989). If DS acts as a ^{15}N point source, we may be able to determine dispersal patterns of fishes using ^{15}N as a tracer. Because surface currents in western Lake Superior are counterclockwise (Lam 1978), the signal from DS should be dispersed along the southern (Wisconsin) coast of the region (Fig. 1). Relatively immobile species should be affected near the point source and gradually return to a natural state at more remote sites along the Wisconsin coast. In contrast, mobile species will integrate the characteristics of many areas and thus be more isotopically homogeneous (Hansson et al. 1997).

The purpose of this study is threefold. First, we use stable isotope analysis to produce a general description of the offshore food web of western Lake Superior. Second, we examine the isotopic signatures of several key taxa at a spatially explicit level to test the hypothesis that anthropogenic nutrients from DS are assimilated into the food web. Third, we use the spatially explicit data to hypothesize fish movement patterns.

Methods

We examined the food web components listed in Table 1. At the base of the food web, we analyzed bulk seston, zooplankton, and two abundant benthic macroinvertebrates (a shrimp, *Mysis relicta*, and an amphipod, *Diporeia hoyi*). Of the fish community, we examined planktivores, both native (lake herring, *Coregonus artedi*), and exotic (rainbow smelt), benthic fishes from <80 m depths (slimy sculpin, *Cottus cognatus*) and >80 m depths (kiyi, *Coregonus kiyi*; bloater, *Coregonus hoyi*; deepwater sculpin, *Myoxocephalus thompsoni*), native predators (lake trout; burbot, *Lota lota*), and exotic salmonids (chinook salmon, *Oncorhynchus tshawytscha*; coho salmon, *Oncorhynchus kisutch*). We subdivided lake trout into two widely recognized races: "lean" lake trout, which live in waters up to ~80 m deep and have relatively low body fat content, and "siscowet" lake trout (*Salvelinus namaycush siscowet*), which live at depths >80 m and have very high body fat (Thurston 1962; Eschmeyer and Phillips 1965; Hansen et al. 1995).

Samples were collected from the western basin of Lake Superior (Fig. 1) during May–September 1997. Seston, zooplankton, and macroinvertebrates were sampled after dark in July throughout the study area (Fig. 1; Table 1). Bulk seston samples were collected in 10-L Niskin bottles at four depths per station: midepilimnion, deep epilimnion, chlorophyll maximum, and hypolimnion. These were determined from measurements taken by a conductivity–temperature–density probe with a fluorometer attachment. Water from the four Niskin bottles was combined into a single 40-L composite and centrifuged for 4 h. We discarded the supernatant, filtered the remaining slurry onto a precombusted glass fiber filter (0.7-μm pore), and froze the filter (–20°C). Zooplankton were collected from 50-m depths to the surface by vertical plankton net (0.5-m-diameter mouth, 63-μm mesh) hauls and then frozen; *Mysis* were manually removed from zooplankton samples, rinsed, and frozen separately. *Diporeia* were collected in benthic grab samples (depth range 50–150 m), rinsed, and frozen. Fishes were collected by trawl and gill net at several sites (Fig. 1; Table 1). Forage species (coregonids, rainbow smelt, sculpins) were collected during May in down-bank bottom trawls that began at depths of ~15 m and ended at depths of 40–100 m. Additional forage fishes were collected in July in midwater trawls or overnight gillnet sets. Piscivores (lake trout, burbot, salmon) were collected in August and September in bottom gill nets set at depths of 20–40 m (lean lake trout, burbot, chinook salmon) and ≥80 m (siscowet lake trout, coho salmon). Fishes were weighed, measured, and frozen.

Samples analyzed for stable isotope composition included seston, zooplankton (sorted into calanoid copepods, cyclopoid copepods, and nonpredatory cladocerans), whole *Mysis* and *Diporeia* (pooled by site), and dorsal white muscle tissues from fishes. Fishes too small to dissect (sculpins, young-of-the-year rainbow smelt) were analyzed whole after removal of the viscera. This method derives from Pinnegar and Polunin (1999), who found that small fish bodies and isolated white muscle tissue exhibited similar isotopic enrichment relative to diet; this result is reasonable, given that white muscle is a major component of small fishes' biomass. Samples were dried to constant mass at 65°C. Animal tissues were ground to a fine powder, and seston filters were acidified by fuming for 2 h with concentrated HCl. Samples were analyzed on a Europa 20/20 continuous-flow mass spectrometer (Horticulture Department, University of Wisconsin-Madison, except for seston filters, which were analyzed at the University of Alaska-Fairbanks). Results are given as δ values, per mil (‰) deviations from standards (atmospheric nitrogen or Pee Dee Belemnite carbon), where

$$\delta^{15}\mathrm{N} \text{ or } \delta^{13}\mathrm{C} = [(R_{\mathrm{SAMPLE}} - R_{\mathrm{STANDARD}})/R_{\mathrm{STANDARD}}] \times 1000 \; (R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N} \text{ or } {}^{13}\mathrm{C}/{}^{12}\mathrm{C}).$$

The δ^{15}N and δ^{13}C values of a consumer should be greater than those of its diet by roughly 3–5 and 0–1‰, respectively, due to isotopic fractionation (Peterson and Fry 1987).

The stable isotope ratios of consumers imply trophic relationships that can be compared with expectations based on previous gut content studies. A simple approach for such comparison is a static mixing model, whereby a consumer's expected δ values are equal to the weighted average δ values of its prey items (weighted by the proportion of each prey item in the gut contents) plus trophic enrichment due to fractionation (Harrigan et al. 1989; Wainright et al. 1993). We estimated the δ^{15}N and δ^{13}C of Lake Superior fishes with such a model, using gut content information from previous efforts and trophic enrichments of +3.4 and +1‰, respectively. Predicted δ values were compared with observed

Fig. 1. Map of western Lake Superior with sampling stations labeled. DS, Duluth–Superior (46°45′N, 92°05′W); M, sites on the Minnesota coast; W, sites on the Wisconsin coast. The prevailing surface current in this region travels counterclockwise from Minnesota to Wisconsin.

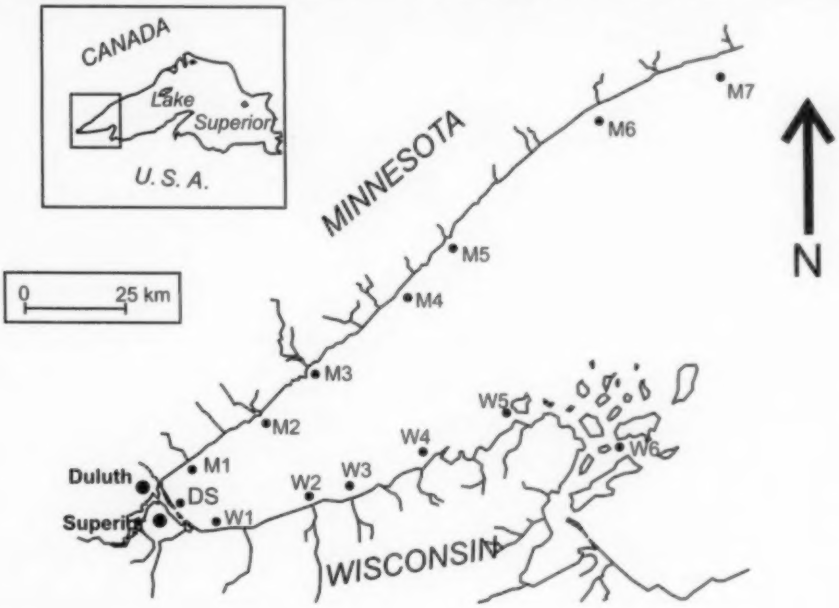


Table 1. Summary of taxa analyzed for stable isotopes in western Lake Superior.

Taxon	Code	Collection sites	Average *n* analyzed per site
Seston	Ses	M7, M3, M2, DS, W1, W3, W5, W6	na
Cladocerans	Cld	M3, M2, DS, W1, W6	192[a]
Calanoids	Cal	M7, M3, M2, DS, W1, W3, W4, W6	258[a]
Cyclopoids	Cyc	M3, M2, DS, W4, W6	752[a]
Diporeia hoyi	Dip	M7, M2, W6	18[a]
Mysis relicta	Mys	M7, M6, M3, M2, W1, W6	10[a]
Lake herring	Her	M7, M2, W1, W2, W4, W6	5
Rainbow smelt	Rsm	M5, M1, DS, W2, W4, W5, W6	6
Slimy sculpin	Sls	M5, M1, DS, W2, W4, W5	8
Deepwater sculpin	Dws	M7	8
Bloater	Whi	W4	10
Kiyi	Kiy	W4	10
Lean lake trout	Llt	M7, M4, M1, W4, W6	5
Siscowet lake trout	Sis	M7, M2, W4, W6	8
Burbot	Bur	M1, DS, W1, W2, W3, W4	8
Chinook salmon	Chs	M1, M2	3
Coho salmon	Cos	M2	3

Note: Collection site codes correspond to those in Fig. 1; na, not available.

[a] Zooplankton and macroinvertebrate samples were pooled at each site.

values via two-tailed *t* tests ($\alpha = 0.05$) in which model predictions served as theoretical mean values.

Of the species sampled, many were not captured at enough sites to evaluate the existence of an isotopic signal originating from DS (Table 1). Taxa for which the spatial resolution of sampling was sufficient to test the DS signal hypothesis were seston, calanoid copepods, *Mysis*, rainbow smelt, lake herring, slimy sculpin, and burbot. Data for fishes were evaluated by a two-factor general linear model (GLM), where the factors were site and total length (to account for possible ontogenetic diet shifts). Seston, calanoid copepods, and *Mysis* values were evaluated qualitatively because those samples were pooled into one composite sample at each site.

Results

Western Lake Superior food web assessment

When stable isotope data from the entire study area were pooled by taxon, the resulting food web diagram showed the expected results of increasing $\delta^{15}N$ and $\delta^{13}C$ from the planktonic community to the large piscivores (Fig. 2). Siscowet lake trout were the top predators, as indicated by their high $\delta^{15}N$. The $\delta^{15}N$ of the other native piscivores, lean lake trout and burbot, was 1–2‰ lighter. Siscowet lake trout $\delta^{13}C$ was similar to that of deepwater coregonids (kiyi, bloater) but

Fig. 2. General food web diagram of western Lake Superior, according to results from stable isotope analysis. Data are mean isotopic concentrations ± 1 SE. Data labels correspond to taxon codes in Table 1.

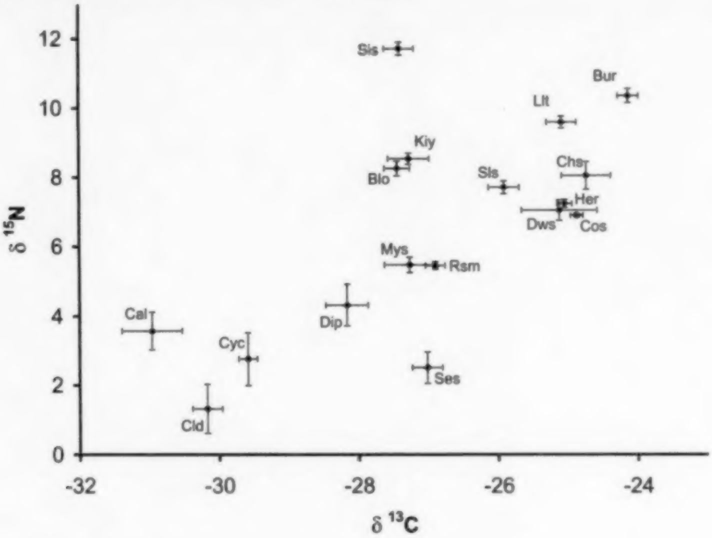


Table 2. Mean predicted and observed stable isotope ratios of Lake Superior fishes.

		$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
Taxon	Gut contents (reference(s))	Predicted	Observed (SE)	Predicted	Observed (SE)
Lake herring	Copepods, cladocerans, *Mysis* (1, 2)	6.86	**7.23 (0.12)**	–28.50	**–25.04 (0.11)**
Rainbow smelt	*Mysis*, copepods, *Diporeia*, cladocerans (1, 2)	7.47	**5.45 (0.11)**	–27.74	**–26.90 (0.14)**
Slimy sculpin	*Diporeia* (3)	7.71	7.70 (0.18)	–27.16	**–25.91 (0.22)**
Deepwater sculpin	*Diporeia*, *Mysis* (3)	8.00	**7.04 (0.29)**	–26.94	**–25.12 (0.54)**
Bloater	*Mysis*, copepods, *Diporeia* (1)	7.71	**8.25 (0.21)**	–27.65	–27.44 (0.18)
Kiyi	*Mysis*, copepods (1)	8.29	8.53 (0.16)	–27.01	–27.27 (0.29)
Lean lake trout	Rainbow smelt, coregonids, sculpins (4, 5, 6)	9.87	9.59 (0.17)	–24.96	–25.08 (0.21)
Siscowet lake trout	Coregonids, sculpins, insects (4, 5, 6)	11.52	11.69 (0.18)	–25.91	**–27.40 (0.21)**
Burbot	Coregonids, rainbow smelt, *Mysis*, sculpins (5)	10.40	10.34 (0.20)	–25.26	**–24.12 (0.15)**
Chinook salmon	Rainbow smelt, coregonids, invertebrates (4)	9.38	**8.04 (0.40)**	–25.38	–24.73 (0.35)
Coho salmon	Rainbow smelt, invertebrates, coregonids (4)	8.86	**6.89 (0.06)**	–25.95	**–24.86 (0.09)**

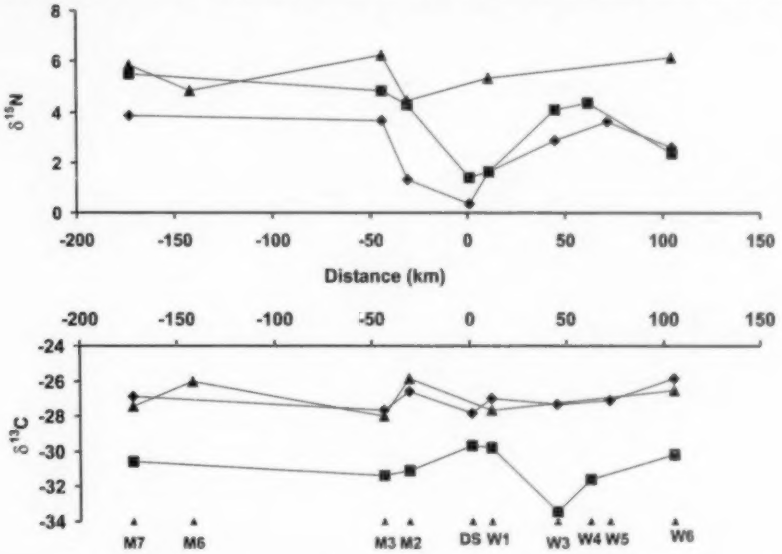
Note: Predicted values are based on empirical gut content analysis (gut contents listed in descending order of importance; components that made up >50% are underlined). Observed values that differed significantly from predicted values are in bold type. References: 1, Anderson and Smith (1971); 2, Johnson et al. (1998); 3, Selgeby (1988); 4, Conner et al. (1993); 5, S. Schram (Wisconsin Department of Natural Resources, Wis., unpublished data); 6, D. Schreiner (Minnesota Department of Natural Resources, Minn., unpublished data).

light relative to species of shallower bathymetric distribution (lean lake trout, burbot, lake herring, slimy sculpin). The $\delta^{15}N$ of the exotic chinook and coho salmon was ~3‰ lighter than that of lean lake trout and burbot. Chinook and coho salmon were isotopically similar to fishes traditionally regarded as forage fishes for top predators, such as lake herring and slimy sculpin.

In the forage fish community, there were substantial differences among the key pelagic species and benthic species. Of the pelagic species, rainbow smelt (adults and young-of-the-year were indistinguishable) were isotopically depleted relative to lake herring (Fig. 2), suggesting that they rely on different prey resources. Of the benthic forage fishes, the two sculpin species had similar $\delta^{15}N$ and $\delta^{13}C$, but deepwater sculpin were enriched in ^{13}C relative to other deepwater fishes (siscowet lake trout, deepwater coregonids). The deepwater coregonids (kiyi, bloater) were enriched in ^{15}N and depleted in ^{13}C compared with the shallower coregonid (lake herring).

Among macroinvertebrates, *Mysis* occupied a higher trophic position than *Diporeia*, according to $\delta^{15}N$ data (Fig. 2). In the planktonic community, seston was isotopically similar or enriched relative to zooplankton. The range of seston $\delta^{15}N$ across the region was large (0.35–3.88‰). The lowest values were in the southwestern part of the region, where chlorophyll concentrations were highest during the sampling period (T. Johnson, Ontario Ministry of Natural Resources, Wheatley, Ont., unpublished data). Thus, the $\delta^{15}N$ of phytoplankton may be close to 0‰, as previously found in this region by Keough et al. (1996), and other materials in the seston may have elevated $\delta^{15}N$ values into the range those of the zooplankton (Fig. 2). Although the signa-

Fig. 3. Spatially explicit $\delta^{15}N$ (top panel) and $\delta^{13}C$ (bottom panel) of seston (◆), calanoid copepods (■), and *M. relicta* (▲) from western Lake Superior. Distances are relative to DS; codes at the bottom correspond to sampling station codes in Fig. 1.



tures among the zooplankton taxa were highly variable, cladocerans consistently had the lowest trophic position of all zooplankton.

The δ values predicted by the static mixing model, based on previous gut content studies, differed significantly from observed values in many cases (Table 2). Among native fishes, differences between predicted and observed values were within ±1‰ for $\delta^{15}N$ but were nonetheless significant for lake herring, deepwater sculpin, and bloater. Several natives (lake herring, sculpins, burbot) had much higher $\delta^{13}C$ than was predicted, and siscowet lake trout had lower $\delta^{13}C$ than expected. For the exotic rainbow smelt and Pacific salmon, $\delta^{15}N$ was always overestimated by the model, and $\delta^{13}C$ was underestimated for both rainbow smelt and coho salmon.

Spatial heterogeneity of isotope signatures

Our hypothesis that food web components close to DS would have greater $\delta^{15}N$ than organisms at more remote areas was not supported by results from the base of the food web. Although there appeared to be differences in $\delta^{15}N$ of the seston, zooplankton, and *Mysis* among sites in western Lake Superior, the pattern of variation did not follow simple expectations. Seston and calanoid $\delta^{15}N$ were lightest in the southern portion of the region close to DS (Fig. 3). *Mysis* $\delta^{15}N$ was highly variable from site to site but exhibited no spatial trend relative to DS.

Among fishes, slimy sculpin showed strong indication of a DS signal (Fig. 4). Slimy sculpin $\delta^{15}N$ was higher at DS than at sites on the Minnesota coast (which are effectively "upstream" of DS because the prevailing surface currents are counterclockwise (Lam 1978)) and then decreased along the Wisconsin coast. Site was the only significant factor ($F_{5,39}$ = 10.42, $p < 0.001$) for this species, so length was removed from the model and the data were analyzed by analysis of variance (ANOVA) with Tukey's post hoc test. At DS and W2, slimy sculpin $\delta^{15}N$ was significantly greater than at other sites ($F_{5,40} = 17.60$, $p < 0.0001$). Similarly, slimy sculpin $\delta^{13}C$ decreased at DS and returned to baseline values quickly (Fig. 4); slimy sculpin $\delta^{13}C$ was lighter at DS than at any other sampled location ($F_{5,40} = 8.54$, $p < 0.0001$).

As with slimy sculpin, burbot $\delta^{15}N$ was greatest at DS (Fig. 4). Burbot $\delta^{15}N$ increased from the "upstream" site (M1) to DS, and the enrichment faded from DS up the Wisconsin coast. However, the GLM analysis revealed that site was not an important factor ($F_{4,33} = 0.64$, $p = 0.63$), but length was highly significant ($F_{1,33} = 10.05$, $p = 0.003$). Reducing the model to a simple regression showed a positive relationship between burbot $\delta^{15}N$ and length (centimetres) (slope = 0.06, $p < 0.001$, $r^2 = 0.36$). Both factors were significant for burbot $\delta^{13}C$ (site: $F_{4,33} = 3.79$, $p = 0.012$; length: $F_{1,33} = 7.61$, $p = 0.009$), as larger burbot tended to have slightly greater $\delta^{13}C$, and burbot at W1, W2, and W4 were more enriched than those at M1 and DS (Fig. 4).

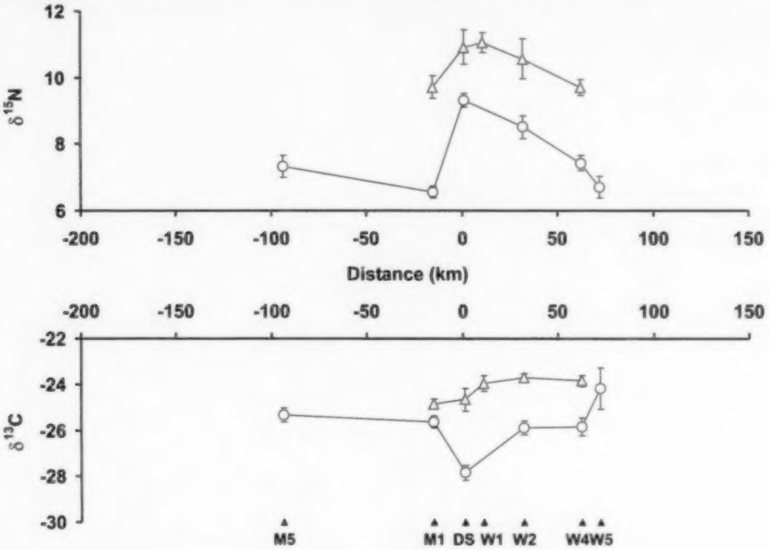
Lake herring $\delta^{15}N$ was variable across the region (Fig. 5), and like slimy sculpin, the GLM yielded only a significant response to site ($F_{5,22} = 5.67$, $p = 0.002$). When the model was reduced to a single-factor ANOVA, values at two sites, W1 and W5, were significantly greater than at the two most remote sites, M7 and W7 ($F_{5,23} = 5.44$, $p = 0.002$). Values at M2 and W2 were intermediate and did not differ significantly from other values. Lake herring showed no systematic variation in $\delta^{13}C$ (GLM, $p > 0.5$ for both factors). Rainbow smelt showed no variation in $\delta^{15}N$ (GLM, $p > 0.1$ for both factors). Rainbow smelt $\delta^{13}C$ was slightly positively related to size ($F_{1,34} = 4.50$, $p = 0.04$) but did not vary spatially ($p > 0.05$).

Discussion

General food web structure

The $\delta^{15}N$ and $\delta^{13}C$ in tissues of Lake Superior organisms imply a food web structure in which siscowet lake trout

Fig. 4. Spatially explicit $\delta^{15}N$ (top panel) and $\delta^{13}C$ (bottom panel) of slimy sculpin (○) and burbot (△) from western Lake Superior. Distances are relative to DS; codes at the bottom correspond to sampling station codes in Fig. 1.



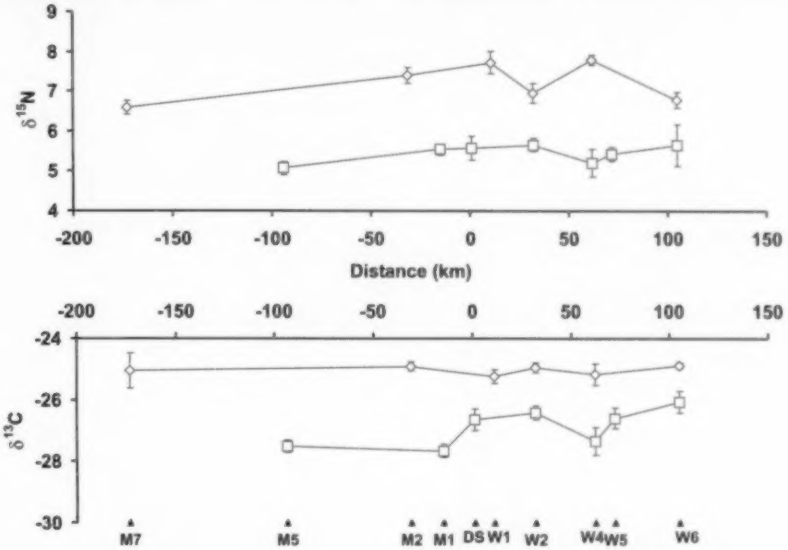
were the top predators in the deepwater community and burbot and lean lake trout were top predators in the nearshore community. However, stable isotope results did not fully complement the results of previous gut content analyses, according to predictions from the static mixing model. Inconsistencies between observations and model predictions may owe to several explanations. First, the gut content data used to generate predicted δ values may not adequately represent the diets of these species. This inadequacy could result from seasonal variation in feeding that is not detected by gut content analyses or by long-term changes in diets that have occurred since the previous gut content studies. Such long-term changes could be caused by ontogenetic diet shifts of consumers or by changes in prey availability related to shifts in community composition. If such variation has occurred, the diets upon which the predictions are based may lack key components that would influence the δ values. Second, temporal variation in stable isotope ratios may occur, particularly in smaller organisms with more rapid tissue turnover rates. Thus, the stable isotope ratios of invertebrates collected in July 1997 may not represent the long-term ratios of the diets of larger consumers. Alternatively, large consumers, with slow growth or tissue turnover rates, probably express isotopic "memory," in that their signatures partly reflect their diets from younger stages of their life histories. The importance of long-term dietary changes among different consumers and temporal variation in tissue isotopic signatures can be resolved by two methods. The first is more intensive temporal sampling of both gut contents and stable isotope ratios. This alternative has obvious empirical value but would be expensive in a large and logistically challenging system like Lake Superior. A second alternative is a dynamic modeling approach, as opposed to a static mixing model approach. Dynamic models, such as the bioenergetics approach of Kitchell et al. (1977), can account for temporal variations in isotopic signatures and diets and could therefore be used to test hypotheses and build inferences about food web linkages in aquatic ecosystems. Such models will be the focus of future research.

A final source of inconsistency between observed and predicted isotope values may be our $\delta^{13}C$ determination methods. For example, we did not extract lipids from our samples before analysis, and lipids, which are depleted in ^{13}C relative to other tissues (McConnaughey and McRoy 1979), may have influenced $\delta^{13}C$ values of some components. Also, our estimated trophic enrichment value for carbon, +1‰, may be incorrect for some or all of the components. DeNiro and Epstein (1978) observed ^{13}C fractionation ranging from –0.6 to +2.7‰, and a reasonable estimate of fractionation from this broad range is needed to determine the congruency of stable isotope and gut content data.

We hesitate to draw conclusions about the diets of invertebrates because our analysis of the planktonic community was rather coarse. For example, we analyzed bulk seston rather than dividing the seston into phytoplankton and detrital components, and we lumped zooplankton into only three functional groups. However, it appears from the isotope data that calanoid copepods, cyclopoid copepods, and cladocerans have distinct diets.

A key result is that the two lake trout races, leans and siscowets, showed little evidence of dietary overlap. Because siscowet lake trout are 10-fold more abundant than lean lake trout and often migrate vertically from their deep habitat to feed in shallower areas (Thurston 1962; Eschmeyer and Phillips 1965; M. Ebener, Chippewa-Ottawa Treaty Fishery Management Authority, Sault-Ste. Marie, Mich., unpublished data), there is concern that diet overlap exists between the two races, which could constrain restoration of lean lake trout populations (Busiahn 1990). Siscowet and lean lake trout $\delta^{13}C$ differed, suggesting that they utilize different pro-

Fig. 5. Spatially explicit $\delta^{15}N$ (top panel) and $\delta^{13}C$ (bottom panel) of lake herring (◇) and rainbow smelt (□) from western Lake Superior. Distances are relative to DS; codes at the bottom correspond to sampling station codes in Fig. 1.



duction bases (but see caveats regarding $\delta^{13}C$ above). Furthermore, siscowet lake trout $\delta^{15}N$ was greater than that of lean lake trout and was enriched by roughly 3.5‰ compared with bloater and kiyi. These data suggest that deepwater coregonids were key components of siscowet lake trout diets. Gut content data for siscowet lake trout corroborate this conclusion. There was no clear isotopic evidence that siscowet lake trout consume significant numbers of rainbow smelt or lake herring. Thus, stable isotope data suggest that trophic overlap between lean and siscowet lake trout in western Lake Superior is not substantial.

Introduced Pacific salmon occupied different trophic positions than native piscivores (lake trout, burbot). Exotic salmon in Lake Superior are a management concern because they are presumed to feed on the same prey resources as native piscivores, and the supply–demand relationship between these predators and the forage base is uncertain (Ebener 1995; Negus 1995). Our results suggest that chinook and coho salmon are lower on the food web than adult lake trout and burbot. If direct foraging competition between exotic and native piscivores exists, it may be between adult salmon and subadult native predators. Exotic salmon appear to rely upon rainbow smelt, which are important prey for young lake trout (Mason et al. 1998), and upon invertebrates, which are important to young lake trout and young burbot (Hansen et al. 1995; S. Schram, Wisconsin Department of Natural Resources, Bayfield, Wis., unpublished data). Adult lake trout and burbot make greater use of coregonids and sculpins than do Pacific salmon, according to our results and gut content analysis. These results illustrate an important application of stable isotope analysis: assessing the role of exotic species in aquatic food webs. Previous studies have used stable isotopes to examine trophic overlap between native and exotic fishes (Gu et al. 1996; Sierszen et al. 1996), trophic shifts in native fishes elicited by invading fishes (Vander Zanden et al. 1999), and the impacts of invading zebra mussels (*Dreissena polymorpha*) on food webs and nutrient cycles (Gardner et al. 1995; Mitchell et al. 1996; Mazak et al. 1997).

Spatial heterogeneity of isotope signatures

Point sources of ^{15}N, such as human population centers, can be useful markers for studying movement patterns of aquatic organisms (Hansson et al. 1997). Our results support this assertion, in that slimy sculpin followed our hypothesized pattern of ^{15}N enrichment near DS that decayed with increasing down-current distance. This result implies that slimy sculpin are relatively sedentary, which is a reasonable conclusion given their benthic nature. It also implies that at least some of the organisms that constitute slimy sculpin diets are also ^{15}N enriched by the DS source. In contrast, our results imply that rainbow smelt are highly vagile in western Lake Superior. The stable isotope ratios of rainbow smelt were similar at all sites, which suggests that rainbow smelt move throughout the region and integrate the isotopic signatures of many different areas.

Some spatial variation in stable isotopes in the Lake Superior fish community could not be attributed to DS. Lake herring $\delta^{15}N$ varied spatially but not in the hypothesized manner due to enrichment by the DS source. This result may indicate that lake herring occur in populations that move across large ranges and thus do not acquire isotopic signatures influenced by a point source such as DS. These ranges, however, are smaller than the range covered by rainbow smelt, and thus, the lake herring isotopic signatures are not homogeneous at the scale we examined. Additionally, burbot $\delta^{15}N$ was heaviest in the DS area, but this was almost certainly caused by ontogenetic diet shifts rather than inputs from DS. Statistical analysis showed that site did not influence burbot $\delta^{15}N$ directly but that length and $\delta^{15}N$ were positively correlated. This illustrates that local variation in size and trophic ontogeny should be considered when examining the spatial patterns of trophic structure and isotopic signatures of a food web. Accounting for ontogenetic variation may clarify vague spatial relationships or conflicting isotope and gut content information (e.g., Wainright et al. 1993).

Many mechanisms underlie spatially explicit isotopic signatures. Two key factors that lead to such spatial variation are (*i*) localized variations in baseline isotope values and (*ii*) the existence of spatially discrete populations of organisms. Variations in isotopic baselines may result from biogeochemical mechanisms such as nutrient contributions from nearby urban sources, wetlands, river basins, or terrestrial runoff (Cabana and Rasmussen 1996; Wainright et al. 1996; Hansson et al. 1997) or possibly from biotic factors such as total primary production or dependence on recycled nutrients (Hecky and Hesslein 1995; Schindler et al. 1997). Spatially discrete populations may then express these localized differences in isotopic baselines, as opposed to highly migratory species that integrate the baselines of many different areas (Hansson et al. 1997). Spatially discrete populations include organisms too small or immobile to move substantially relative to the scale of the study region (e.g., slimy sculpin) and those that exist in distinct local stocks, as has been suggested of Lake Superior lake herring (Bronte et al. 1996) and lean lake trout (Hansen et al. 1995). As illustrated earlier by data for burbot, local variations in size distributions and trophic ontogeny of consumers also contribute to spatial variation in isotopic signatures. Furthermore, tissue turnover rates of different organisms may confound spatial analysis. For example, the isotopic signatures of seston, zooplankton, and *Mysis* varied considerably across the region, but the patterns of variation did not follow our hypothesis concerning the influence of DS. Small organisms such as these experience faster tissue turnover than larger organisms, such as fish, and therefore have greater temporal variability in isotope signatures (e.g., Cabana and Rasmussen 1996). This temporal variation is thus superimposed upon any spatial differences that may exist among these small taxa and, of course, upon the differences owing to water mass dynamics.

In conclusion, our study demonstrates the utility of stable isotope analysis in evaluating fish community interactions, examining the roles of exotic species, and determining general movement patterns of organisms relative to an isotopic point source. Given the continuously changing nature of this system, however, we are compelled to note that our results on western Lake Superior's food web structure and spatial nature represent the current state of the Lake Superior fish community. Changes in production and recruitment dynamics of fishes, stocking of exotic species, and management practices focused on restoring other native species (e.g., coaster brook trout, *Salvelinus fontinalis*, and lake sturgeon, *Acipenser fulvescens*) can affect the abundances of food web components through time. Such changes may lead to seasonal or long-term dietary shifts that fundamentally affect isotopic signatures, particularly of rapidly growing species (e.g., Wainright et al. 1993; Cabana and Rasmussen 1994; Yoshioka et al. 1994). Furthermore, our results are based on adult stages for most fishes, but interspecific dietary overlap may differ considerably over the course of the species' full life histories. Thus, future research will include the examination of ontogenetic diet shifts in key food web components and the development of dynamic food web models that incorporate stable isotopes in an approach similar to that used in bioenergetics modeling. Development of this tool would enable us to evaluate future impacts of community changes on the trophic dynamics of this system.

Acknowledgements

Field assistance was provided by the U.S. Geological Survey (Lake Superior Biological Station, Ashland, Wis.), the Wisconsin and Minnesota Departments of Natural Resources, and the Environmental Protection Agency (Mid-Continent Ecology Division, Duluth, Minn.). Comments by D. Mason, T. Johnson, M. Sierszen, S. Hansson, K. Schulz, T. Essington, D. Post, W. Porter, G. Cabana, and two anonymous reviewers greatly improved this manuscript. Most samples were run by A. Krueger; other laboratory assistance was provided by N. Haubenstock, K. Hammarsten, A. Cotter, and V. Brady. This study was funded by the University of Wisconsin Sea Grant Institute under grants from the U.S. Department of Commerce, National Oceanic and Atmospheric Administration Sea Grant (grants NA46RG0481 and NA86RG0047), and the State of Wisconsin and by the Anna Grant Birge Memorial Fund.

References

Anderson, E.D., and Smith, L.L. 1971. A synoptic study of food habits of 30 fish species from western Lake Superior. Minn. Agric. Exp. Stn. Rep. No. 279.

Bronte, C.R., Hesselberg, R.J., Shoesmith, J.A., and Hoff, M.H. 1996. Discrimination among spawning concentrations of Lake Superior lake herring based on trace element profiles in sagittae. Trans. Am. Fish. Soc. **125**: 852–859.

Busiahn, T.R. 1990. Fish community objectives for Lake Superior. Great Lakes Fish. Comm. Spec. Publ. No. 90-1.

Cabana, G., and Rasmussen, J.B. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature (Lond.), **372**: 255–257.

Cabana, G., and Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. U.S.A. **93**: 10 844 – 10 847.

Christie, W.J. 1974. Changes in the fish species composition of the Great Lakes. J. Fish. Res. Board Can. **31**: 827–854.

Conner, D.J., Bronte, C.R., Selgeby, J.H., and Collins, H.L. 1993. Food of salmonine predators in Lake Superior, 1981–1987. Great Lakes Fish. Comm. Tech. Rep. No. 59.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**: 495–506.

Ebener, M.H. 1995. Bioenergetics of predatory fish in western US waters of Lake Superior. Report prepared for the Red Cliff Band of Lake Superior Chippewas. Red Cliff Fisheries Department, Bayfield, Wis.

Eschmeyer, P.H., and Phillips, A.M., Jr. 1965. Fat content of the flesh of siscowets and lake trout from Lake Superior. Trans. Am. Fish. Soc. **94**: 62–74.

Gardner, W.S., Cavaletto, J.F., Johengen, T.H., Johnson, J.R., Heath, R.T., and Cotner, J.B., Jr. 1995. Effects of the zebra mussel, *Dreissena polymorpha*, on community nitrogen dynamics in Saginaw Bay, Lake Huron. J. Gt. Lakes Res. **21**: 529–544.

Gu, B., Schelske, C.L., and Hoyer, M.V. 1996. Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow eutrophic lake. J. Fish Biol. **49**: 1233–1243.

Hansen, M.J., et al. 1995. Lake trout (*Salvelinus namaycush*) populations in Lake Superior and their restoration in 1959–1993. J. Gt. Lakes Res. **21**(Suppl. 1): 152–175.

Hansson, S., Hobbie, J.E., Elmgren, R., Larsson, U., Fry, B., and Johansson, S. 1997. The stable isotope ratio as a marker of food-web interactions and fish migration. Ecology, **78**: 2249–2257.

Harrigan, P., Zieman, J.C., and Macko, S.A. 1989. The base of nutritional support for the gray snapper (*Lutjanus griseus*): an evaluation based on a combined stomach content and stable isotope analysis. Bull. Mar. Sci. **44**: 65–77.

Hecky, R.E., and Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J. North Am. Benthol. Soc. **14**: 631–653.

Johnson, T.B., Mason, D.M., Bronte, C.R., and Kitchell, J.F. 1998. Estimation of invertebrate production from patterns of fish predation in western Lake Superior. Trans. Am. Fish. Soc. **127**: 496–506.

Keough, J.R., Sierszen, M.E., and Hagley, C.A. 1996. Analysis of a Lake Superior coastal food web with stable isotope techniques. Limnol. Oceanogr. **41**: 136–146.

Kidd, K.A., Schindler, D.W., Muir, D.C.G., Lockhart, W.L., and Hesslein, R.H. 1995. High concentration of toxaphene in fishes from a subarctic lake. Science (Washington, D.C.), **269**: 240–242.

Kiriluk, R.M., Servos, M.R., Whittle, D.M., Cabana, G., and Rasmussen, J.B. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Can. J. Fish. Aquat. Sci. **52**: 2660–2674.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. **34**: 1922–1935.

Kling, G.W., Fry, B., and O'Brien, W.J. 1992. Stable isotopes and planktonic structure in arctic lakes. Ecology, **73**: 561–566.

Lam, D.C.L. 1978. Simulation of water circulations and chloride transports in Lake Superior for summer 1973. J. Gt. Lakes Res. **4**: 343–349.

Mason, D.M., Johnson, T.B., and Kitchell, J.F. 1998. Consequences of prey fish community dynamics on lake trout foraging efficiency in Lake Superior. Can. J. Fish. Aquat. Sci. **55**: 1273–1284.

Mazak, E.J., MacIsaac, H.J., Servos, M.R., and Hesslein, R. 1997. Influence of feeding habits on organochlorine contaminant accumulation in waterfowl on the Great Lakes. Ecol. Appl. **7**: 1133–1143.

McConnaughey, T., and McRoy, C.P. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar. Biol. **53**: 257–262.

Mitchell, M.J., Mills, E.L., Idrisi, N., and Michener, R. 1996. Stable isotopes of nitrogen and carbon in an aquatic food web recently invaded by *Dreissena polymorpha* (Pallas). Can. J. Fish. Aquat. Sci. **53**: 1445–1450.

Negus, M.T. 1995. Bioenergetics modeling as a salmonine management tool applied to Minnesota waters of Lake Superior. N. Am. J. Fish. Manage. **15**: 60–78.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**: 293–320.

Pinnegar, J.K., and Polunin, N.V.C. 1999. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. Funct. Ecol. **13**: 225–231.

Rathke, D.E., and McRae, G. (*Editors*). 1989. 1987 report on Great Lakes water quality. Appendix B. Great Lakes surveillance. Vol. I. International Joint Commission, Windsor, Ont.

Schindler, D.E., Carpenter, S.R., Cole, J.J., Kitchell, J.F., and Pace, M.L. 1997. Influence of food web structure on carbon exchange between lakes and the atmosphere. Science (Washington, D.C.), **277**: 248–251.

Selgeby, J.H. 1988. Comparative biology of the sculpins of Lake Superior. J. Gt. Lakes Res. **14**: 44–51.

Sierszen, M.E., Keough, J.R., and Hagley, C.A. 1996. Trophic analysis of ruffe (*Gymnocephalus cernuus*) and white perch (*Morone americana*) in a Lake Superior coastal food web, using stable isotope techniques. J. Gt. Lakes Res. **22**: 436–443.

Thurston, C.E. 1962. Physical characteristics and chemical composition of two subspecies of lake trout. J. Fish. Res. Board Can. **19**: 39–44.

Vander Zanden, M.J., Casselman J.M., and Rasmussen J.B. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature (Lond.), **401**: 464–467.

Wainright, S.C., Fogarty, M.J., Greenfield, R.C., and Fry, B. 1993. Long-term changes in the Georges Bank food web: trends in stable isotopic compositions of fish scales. Mar. Biol. **115**: 481–493.

Wainright, S.C., Fuller, C.M., Michener, R.H., and Richards, R.A. 1996. Spatial variation of trophic position and growth rate of juvenile striped bass (*Morone saxatilis*) in the Delaware River. Can. J. Fish. Aquat. Sci. **53**: 685–692.

Weiler, R.R. 1978. Chemistry of Lake Superior. J. Gt. Lakes Res. **4**: 370–385.

Yoshioka, T., Wada, E., and Hayashi, H. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. Ecology, **75**: 835–846.

Biomass transport to and from an upper estuarine area by migration of juvenile Atlantic herring *Clupea harengus*

Joachim Maes and Frans Ollevier

Abstract: A model describing fish abundance resulting from fish migration and mortality in an upper estuary is presented. This model can be used to estimate biomass transports by fish migrations between estuary and sea and to assess production assimilated in the estuary. It was applied to herring *Clupea harengus* L., a marine species with 0+ juveniles that migrate during winter to temperate European estuaries. It was shown that different mortality regimes greatly affected the number of fish that eventually emigrated and, hence, the biomass that was exported during the seaward migration. The difference between imported and exported biomass was assessed under different growth conditions and varied from positive to negative as mortality rates increased. The discrepancy between export of biomass and in situ produced biomass showed that fish production was not necessarily transported to the sea when emigrating. It was tentatively concluded that export of biomass out of the estuary only occurs if populations move seaward before winter.

Résumé : Nous présentons un modèle qui décrit l'abondance des poissons dans un estuaire en considérant la migration et la mortalité. Ce modèle peut être utilisé pour, d'une part, estimer le transport de biomasse entre les estuaires et la mer, et d'autre part estimer la production assimilée par l'estuaire lors de la migration des différentes populations. Le modèle a été, au cours de nos travaux, appliqué sur le hareng *Clupea harengus* L., une espèce marine dont les juvéniles 0+ migrent vers les estuaires Européens pendant l'hiver. Nos résultats montrent que les différents régimes de mortalité au sein de l'estuaire, influencent grandement le nombre de poissons qui émigrent et la biomasse exportée vers la mer. L'exportation de biomasse a été estimée sous différentes conditions de croissance dépendantes du taux de mortalité. La différence entre la biomasse exportée et la biomasse produite in situ nous permet de montrer que la production de poissons n'est pas toujours exportée lors de l'émigration. Nous avons donc provisoirement conclue que l'exportation de biomasse n'a lieu que lorsque les populations migrent vers la mer avant l'hiver.

Introduction

Marine fish and crustaceans often migrate to shallow turbid coastal areas and estuaries during the first stages of their life to join highly productive feeding habitats and to profit from enhanced protection from predators (Haedrich 1983; Day et al. 1989; Blaber 1997). However, few researchers have focussed on the transport of energy and biomass between estuaries and coastal marine ecosystems by fish migrations (Deegan 1993). This contrasts with the rather well-developed export budgets available for nutrients and plankton (Heip and Herman 1995). The number of studies estimating export of fish biomass and fish production is limited because the complex migration patterns make it difficult to use the standard methods (Day et al. 1989). The purpose of this paper is to present a model that describes fish abundance in an estuary as a result of migration and mortality. The second objective is to use the model to approximate the amount of biomass that is either imported into an estuary or exported seaward by a migrating population. The model is applied to Atlantic herring, *Clupea harengus* L., a marine species with young-of-the-year that commonly inhabit the brackish-water area of North Sea estuaries.

Materials and methods

Model of fish abundance, biomass transfers, and fish production

The change in fish abundance (N) over time (t) in the estuary as the result of migration and mortality can be expressed as

$$(1)\qquad \frac{dN}{dt} = \text{immigration} - \text{emigration} - \text{mortality}$$

Equation 1 is a conceptual model describing fish abundance over time. One biological process, immigration, is responsible for increasing fish abundance (source). Two biological processes, mortality and emigration, are responsible for decreasing fish abundance (sinks). Given that both migration and mortality have an exponential course, eq. 1 can be rewritten in a mathematical form:

$$(2)\qquad \frac{dN}{dt} = a \cdot \exp(it) - e \cdot N - z \cdot N$$

where a, i, e, and z are the parameters of the model. All symbols used in equations and text are explained in Table 1. Equation 2 is a differential equation with dependent variable N and independent variable t. As immigration and emigration were assumed to be discontinuous with time, eq. 2 has to be split into two parts to find a proper solution. Between t_0 and $t_{\max}$, eq. 2 can be shortened (eq. 3)

Received August 17, 1999. Accepted April 14, 2000.
J15317

J. Maes[1] and F. Ollevier. Katholieke Universiteit Leuven, Laboratory of Aquatic Ecology, Ch. De Beriotstraat 32, B-3000 Leuven, Belgium.

[1] Author to whom all correspondence should be addressed. e-mail: joachim.maes@bio.kuleuven.ac.be

Table 1. Symbols used in the equations and in the text.

Symbol	Explanation	Unit
$N(t)$	Fish abundance at time t	Number/10^3 m^3
$N_i(t)$	Fish abundance during immigration	Number/10^3 m^3
$N_e(t)$	Fish abundance during emigration	Number/10^3 m^3
t	Time	Day
t_0	Time of hatching	Day
t_{max}	Time of maximum abundance	Day
t_{end}	Time by which emigration is ended	Day
a	Intercept with dN/dt	Number/10^3 m^3/day
i	Immigration coefficient	day^{-1}
e	Emigration coefficient	day^{-1}
z	Total mortality coefficient	day^{-1}
$L(t)$	Length at age t	mm
$W(t)$	Weight at age t	g
$L_{infinity}$	Asymptotic length	mm
$W_{infinity}$	Asymptotic weight	g
K	Growth coefficient	day^{-1}
I	Number of fish immigrated	Number/10^3 m^3
E	Number of fish emigrated	Number/10^3 m^3
B_i	Imported biomass	g/10^3 m^3
B_e	Exported biomass	g/10^3 m^3
P	Production	g/10^3 m^3/year

and solved for N_i with t as independent variable and $N_i(0) = 0$ (or at t_0, no fish are present yet) as initial condition (eq. 4).

$$(3) \qquad \frac{dN_i}{dt} = a \cdot \exp(it) - z \cdot N_i$$

$$(4) \qquad N_i = \frac{a}{i+z}[\exp(it) - \exp(-zt)]$$

Between t_{max} and t_{end}, eq. 2 can be shortened (eq. 5). Solving eq. 5 for N_e with t as independent variable and with the initial condition that $N_i(t_{max})$ should equal $N_e(t_{max})$ gives eqs. 6 and 7. In eq. 7, the first factor is replaced by $N_i(t_{max})$.

$$(5) \qquad \frac{dN_e}{dt} = -e \cdot N_e - z \cdot N_e$$

$$(6) \qquad N_e = \frac{a}{i+z}[\exp(it_{max}) - \exp(-zt_{max})] \times \exp(-(z+e)(t - t_{max}))$$

$$(7) \qquad N_e = N_i(t_{max}) \cdot \exp(-(z+e)(t - t_{max}))$$

Equations 4 and 7 are analytical functions explaining increasing fish abundance during a period of immigration and decreasing fish abundance during a period of emigration, respectively. These equations, combined with growth data, can now be used to calculate biomass transports. Growth may be expressed by a Von Bertalanffy growth function (Von Bertalanffy 1938) for length (eq. 8) or for biomass (eq. 9):

$$(8) \qquad L(t) = L_{infinity}[1 - \exp(-K(t - t_0))]$$

$$(9) \qquad W(t) = W_{infinity}[1 - \exp(-K(t - t_0))]^3$$

We now can calculate successively the number of fish that have immigrated, the imported biomass, the number of fish that have emigrated, the exported biomass, and the production or the biomass assimilated in the estuary. Integrating eq. 3 from 0 to t_{max} with the mortality coefficient z set to zero yields the number of fish I that have immigrated to the estuary (eq. 10). Equation 10 combined with eq. 9 gives the imported biomass B_i (eq. 11).

$$(10) \qquad I = \int_0^{t_{max}} a \cdot \exp(it)dt = \frac{a}{i}\exp(it_{max}) - \frac{a}{i}$$

$$(11) \qquad B_i = \int_0^{t_{max}} a \cdot \exp(it) \cdot W(t)dt$$

Accordingly, the number of fish E that have emigrated is given by eq. 12. Combining eq. 12 with eq. 9 gives the exported biomass B_e (eq. 13).

$$(12) \qquad E = \int_{t_{max}}^{t_{end}} e \cdot N_e dt = \frac{e}{e+z} N_i(t_{max}) \times [1 - \exp \times ((e+z)(t_{max} - t_{end}))]$$

$$(13) \qquad B_e = \int_{t_{max}}^{t_{end}} e \cdot N_e \cdot W(t)dt$$

We calculated fish production P or the biomass assimilated between t_0 and t_{end} according to eq. 3 of Mertz and Myers (1998) as the product of fish abundance and growth rate (Allen method).

$$(14) \qquad P = \int_{t_0}^{t_{end}} N_i(t)\frac{dW(t)}{dt}dt + \int_{t_{max}}^{t_{end}} N_e(t)\frac{dW(t)}{dt}dt$$

Fish production is thus the amount of tissue elaborated per unit time per unit area, regardless of its fate (Clark 1946). In contrast, transported biomass is the amount of tissue per unit area that is either imported or exported by living animals.

Field sampling

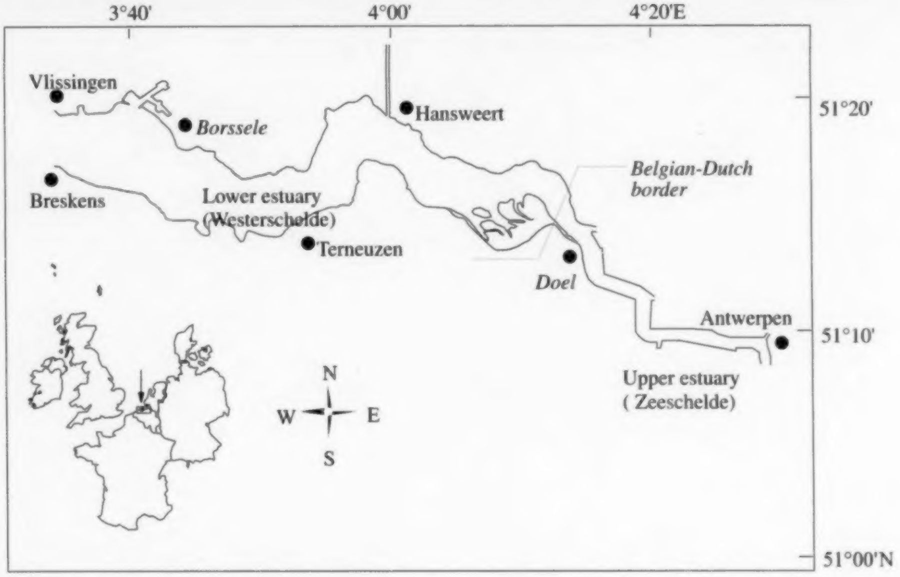
Between July 1994 and June 1995, herring were collected every 2 weeks on the cooling-water filter screens of the nuclear power plant Doel, situated in the Schelde Estuary (Belgium). Approximately 8 × 10^6 m^3 of cooling water was monitored in 135 samples. Mean salinity at the sampling site was 8‰. A full description of the sampling site and sampling method is given in Maes et al. (1998). Number per sample were transformed to number per 10^3 m^3 cooling water sampled. Afterwards, mean abundance for each sampling day was computed and used for further analysis. Biomass of herring was calculated using a length–weight regression (N = 168, R^2 = 0.97, $p < 0.01$): wet weight (g) = exp[−10.529 + 2.6889ln(total length (mm))].

During the same period, KEMA Power Generation & Sustainables collected fish on the filter screens of the nuclear power plant Borssele, situated downstream of Doel near the mouth of the estuary (The Netherlands) (De Potter and Van Aerssen 1995). Salinity at Borssele varied between 18‰ and 35‰. Although the sampling method at Borssele was identical with ours, only 1 × 10^6 m^3 cooling water was monitored in 48 samples taken every month. Fish density at Borssele was generally lower than at Doel because of the sheltered position of the cooling-water intake after dams; therefore, data from Borssele were considered to be of indicative value. The sampling sites are presented in Fig. 1.

Growth and mortality parameters used in the model

Mortality rates were obtained from Pauly (1982) and ranged from 0·day^{-1} to 0.05086·day^{-1}. Higher mortality rates contradicted the field data. Back-extrapolation of the length–frequency modes of herring yielded t_0 (Titmus et al. 1978). Time of hatching was 6

Fig. 1. Map showing the Schelde Estuary and the sampling sites Borssele and Doel.

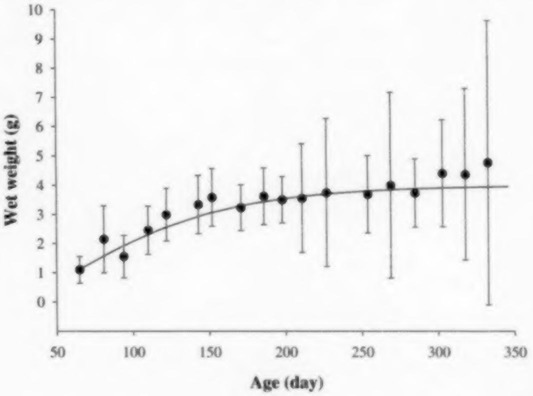


May 1994. We used two growth models to calculate production and transported biomass. The first model used growth parameters K and W_{infinity}, estimated by fitting eq. 9 through our own biomass-at-age data (Fig. 2, Table 2). These data suggested rapid growth during summer and fall and slow growth in winter. The maximum biomass reached after the first growing season was taken as start value for W_{infinity} because the herring population consisted only of young-of-the-year. The second growth model used growth parameters taken from a North Sea herring population (Pauly 1982) (W_{infinity} = 200 g; $K = 0.0011 \cdot \text{day}^{-1}$).

Statistical analysis and mathematical calculations

Non-linear estimation procedures were used to fit eqs. 4 and 7 through the observed abundance data. We used the quasi-Newton method as algorithm for estimating the different model parameters. The loss function used was the least squares estimation method. Explained variance by the fitting was expressed by R^2. All integrals were solved in Mathematica 3.0.

Fig. 2. Mean weight-at-age for herring (●) caught on the cooling-water intake screens at Doel. Error bars represent standard deviations. A non-linear regression (eq. 9) was used compute growth parameters. $W(t) = 3.99(1 - \exp(-0.0163t))^3$ ($R^2 = 0.84$, $p < 0.01$).



Results

By comparing the annual pattern of capture of herring at Doel in the upper estuary with data for Borssele in the lower estuary, the movement of 0-group herring can be inferred (Henderson and Holmes 1989). The proportion of the annual catch caught in each month at Doel and Borssele is shown in Fig. 3. Abundance of herring peaked first at Borssele in November, after which numbers declined because of the movement of fishes to the upper estuary. At Doel, maximum abundance was reached in December. By the end of December, the fish retreated seaward yielding another abundance maximum at Borssele in February. By April the fish had almost completed their seaward migration.

Herring abundance at Doel (Table 2) was modelled using eqs. 4 and 7 (Fig. 4). Using nine different values for mortality (z), the model parameters a, i, and e were estimated (Table 3). Increasing mortality rates caused decreasing emigration rates and increased the value for the intercept a. The rate of immigration remained constant at $0.05 \cdot \text{day}^{-1}$.

The model was used to estimate the number of fish that immigrated (eq. 10) and emigrated (eq. 12) as well as their biomass transported during migration (eqs. 11 and 13, respectively) (Table 3). As mortality increased, the number of fish that immigrated into the brackish water area and the imported biomass had to increase to maintain the maximal fish abundance as observed in the field. Accordingly, the number of fish that moved seaward as well as the exported biomass from the upper estuarine site decreased.

The difference between the imported biomass and exported biomass decreased as mortality rates increased. This relationship is presented in Fig. 5. The growth model that used growth parameters of the North Sea herring stock resulted in

Table 2. Field observations on the presence of Atlantic herring in the upper Schelde Estuary including sampling date (day/month/year), age (days), mean fish abundance (*N*), and average length-at-age (*L*(*t*)).

Sampling date	Age	*N*	*L*(*t*)
11/07/1994	65	0.007	50.88
27/07/1994	81	0.409	64.4
09/08/1994	94	0.169	57.6
25/08/1994	110	0.085	69.17
06/09/1994	122	2.222	75.04
27/09/1994	143	5.627	77.72
06/10/1994	152	7.912	78.79
25/10/1994	171	13.262	75.58
09/11/1994	186	19.705	78.79
21/11/1994	198	138.709	78.23
04/12/1994	211	174.285	77.93
20/12/1994	227	55.811	78.38
16/01/1995	254	49.407	79.84
30/01/1995	268	5.725	81.79
16/02/1995	285	38.37	80.17
06/03/1995	301	2.431	84.45
22/03/1995	319	9.407	83.94
05/04/1995	333	1.054	84.88
18/04/1995	346	1.038	90.84

a lower biomass import and export under different mortality regimes than the growth model that used growth parameters estimated from field data (Fig. 5). Likewise, recruitment, which was interpreted as the percentage of (living) individuals emigrating seaward relative to the number of fish immigrating, decreased as mortality rates increased. Without mortality, recruitment was 100%; maximal mortality yielded zero recruitment as all fish that immigrated eventually died (Table 3).

The estimated fish production computed using eq. 14 differed between the two growth models. When growth parameters were estimated from field measurements, the fish biomass produced by the herring population when present in the upper estuary was 46.3 g wet weight per 10^3 m^3 per year. Using growth parameters from the North Sea herring population stock, total production amounted to 145.7 g wet weight per 10^3 m^3 per year.

Discussion

The present formulation of fish abundance at an upper estuarine site as a result of migration and mortality implied three major assumptions: (*i*) there was no temporal overlap between immigration and emigration; (*ii*) the mortality rate was assumed not to vary with time; and (*iii*) immigration and emigration occur at an exponential rate.

The first hypothesis cannot be confirmed or rejected by our observations. However, the pattern of estuarine movements as revealed by Fig. 2 suggests that this assumption appears to be valid. Analysis of the stable isotopes of carbon and nitrogen in herring tissue can distinguish between newly arrived herring and herring that have been present for some time in the estuary (Hobson 1999). Using this technique would reveal if this assumption is legitimate. These results are not available yet.

Fig. 3. The annual intake pattern of Atlantic herring in the upper Schelde Estuary at Doel (○) and the lower Schelde Estuary at Borssele (●).

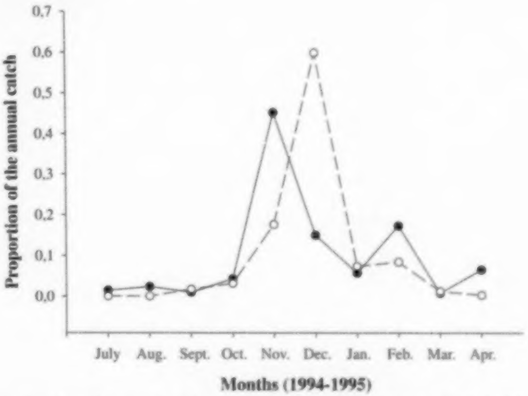
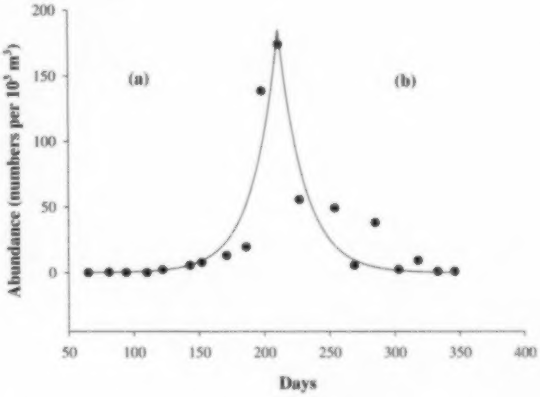


Fig. 4. Abundance of Atlantic herring (●) in the upper Schelde Estuary during immigration (*a*) and emigration (*b*) as determined from cooling-water screen catches at Doel. Fish abundance was fitted (*a*) during immigration by eq. 4 and (*b*) during emigration by eq. 7. Maximum abundance was reached at t_{max} = day 211; emigration was completed at t_{end} = day 346. Explained variance by the fitting (R^2) was 0.918 for immigration and 0.881 for emigration ($p < 0.01$).



The second hypothesis assumed that the mortality rate was constant. With increasing fish size, however, this rate may change (King 1995; De Barros and Toresen 1998). However, major changes in the natural mortality rate of herring visiting the upper estuary at Doel were not expected for three reasons. Firstly, juvenile herring all survived their first summer at sea and were about to recruit to the age-1 group. Secondly, the majority of herring recorded at Doel were only present for a relatively short period of time. Thirdly, piscivorous fish (gadoids) avoided the upper estuary because turbidity levels were unsuitable for visual predation on herring (Maes et al. 1998).

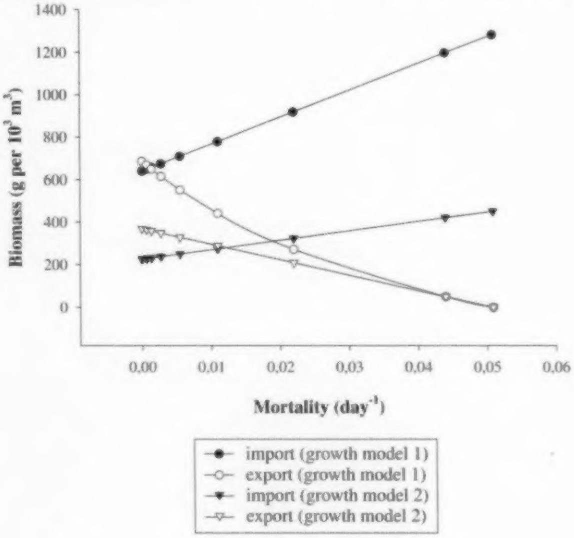
The third hypothesis assumed an exponential increase and decrease in fish abundance as the result of fish migration. Basically, this assumption questions why fish use estuaries and what factors control migration behaviour. Both environmental variations and biological interactions may cause

Table 3. Model parameters estimated by a non-linear estimation procedure.

z	a	i	N_{max}	e	I	E	B_i (1)	B_e (1)	B_i (2)	B_e (2)
0	0.000202	0.050964	185.45	0.050864	185.4	185.3	640.8	686.5	225.2	367.7
0.000686	0.000207	0.050916	185.80	0.050177	188.3	183.1	650.7	669.3	228.6	363.4
0.001373	0.000209	0.050927	185.52	0.049490	190.5	180.3	658.4	650.4	231.3	357.9
0.002746	0.000216	0.050888	185.43	0.048117	195.4	175.2	675.3	615.7	237.2	347.8
0.005493	0.000225	0.050938	185.53	0.045370	205.5	165.3	710.3	552.2	249.6	328.1
0.010987	0.000247	0.050932	185.39	0.039876	225.4	145.2	778.8	441.3	273.6	288.2
0.021975	0.000290	0.050954	185.66	0.028888	265.7	105.3	918.3	271.2	322.7	209.1
0.043950	0.000379	0.05094	185.93	0.006913	346.4	25.2	1196.9	49.8	420.5	50.1
0.050864	0.000408	0.050913	185.56	0.000000	370.9	0.0	1281.9	0.0	450.3	0.0

Note: Equation 4 was used to estimate the intercept *a* and the immigration coefficient *i* under different mortality regimes. Equation 7 was used to estimate the emigration coefficient *e*. Import and export of biomass *B* was calculated using two different growth models: (1) growth parameters from field observations; (2) growth parameters from Pauly (1982).

Fig 5. The relationship between mortality rate and transport of biomass by the Atlantic herring population. Two different growth models were used. Growth model 1 was as presented in Fig 2. Growth model 2 used growth constants from the North Sea herring population (Pauly 1982) ($W_{infinity}$ = 200 g; K = 0.0011·day^{-1}).



migrations of fish upstream or downstream. Large-scale experiments discriminating between factors controlling the movement of marine fish to an estuary are difficult to deploy. Ecological cost–benefit models, however, may predict seasonal usage of an estuary by fish. For example, the downstream movement of young shad in the Hudson River was delayed by the presence of predators in the lower estuary. Decreasing temperatures and food resources forced the fish to move downstream, even in the face of a high predation risk (Limburg 1996). Increased size-related predation at sea or temperature and salinity tolerance limits may cause exponential increase or decrease in abundance. Further confidence for exponential migration rates was given by the fact that large increases of juvenile herring and sprat during winter months have been observed in other North Sea estuaries as well (Van Den Broek 1979; Wharfe et al. 1984; Elliott et al. 1990; Henderson et al. 1992).

The use of a second growth model obtained from the North Sea herring population (Pauly 1982) was necessary as the average length of herring measured at Doel may be biased because of the entrapment of smaller individuals (Henderson et al. 1992), which could cause the marked slow down of growth in November and December. In addition, no reliable estimate of the maximum weight $W_{infinity}$ could be obtained as the samples consisted almost exclusively of age-0 juveniles.

It was shown that different mortality regimes greatly affected the number of fish that eventually emigrated and, hence, the biomass that was transported during the seaward migration. The difference between import and export was assessed under different growth conditions and varied from positive to negative as mortality rates increased. If growth parameters from the North Sea herring stock were used, import equalled export at a mortality of z = 0.012·day^{-1}. Using growth parameters estimated from field observations, import balanced export at a mortality rate z = 0.0012·day^{-1}.

A weakness of this study was that mortality rates for the Schelde Estuary herring population were not available. As a result, we used nine different mortality rates ranging from zero mortality to complete extinction. Under the assumption that the difference between two abundance peaks recorded at Borssele in November and February was caused only by mortality and not by sampling bias, the mortality rate for the herring population was estimated at 0.011·day^{-1}. At this rate of mortality, approximately 65% of the fish that immigrated were able to move out of the upper estuarine region while 35% died in the estuary (Table 3).

Given the differences between export biomass and in situ produced biomass, it is apparent that fish production at an estuarine site is not necessarily transported to the sea when emigrating. This observation contradicts the findings of Deegan (1993), who estimated that average net biomass exported by *Brevoortia patronus* from a Louisiana estuary to the Gulf of Mexico was 38 g·m^{-2}·year^{-1}, corresponding to 5–10% of the total primary production. Unlike herring, *Brevoortia patronus* occupied the estuary between February and November, resulting in net export of biomass. It is thus tentatively concluded that export significantly exceeds import if populations move seaward before winter.

Acknowledgements

R. Hadderingh kindly gave permission for use of data from KEMA. The authors thank A. Taillieu for sampling support and G. Verbouwe, Dr. F. Volckaert, P. Puelinckx, and I. De Saedeleer for mathematical advice. Dr. K. Soetaert substantially improved the paper by assisting with the modelling. Electrabel Doel and the F.W.O. Vlaanderen have funded this study.

References

Blaber, S.J.M. 1997. Fish and fisheries of tropical estuaries. Fish and Fisheries Series No. 22. Chapman and Hall, New York.

Clark, G.L. 1946. Dynamics of production in a marine area. Ecol. Monogr. **16**: 321–335.

Day, J.W., Hall, C.A.S., Kemp, W.M., and Yáñez-Arancibia, A. 1989. Estuarine ecology. Wiley, New York.

De Barros, P., and Toresen, R. 1998. Variable natural mortality rate of juvenile Norwegian spring-spawning herring (*Clupea harengus* L.) in the Barents Sea. ICES J. Mar. Sci. **55**: 430–442.

Deegan, L.A. 1993. Nutrient and energy transport between estuaries and coastal marine ecosystems by fish migration. Can. J. Fish. Aquat. Sci. **50**: 74–79.

De Potter, M.R., and Van Aerssen, G.H.F.M. 1995. Impingement of fish, crabs, shrimps and jellyfish by the power plant Borssele 1994–1995. KEMA, 6812 AR Arnhem, The Netherlands. (Translated from Dutch.)

Elliott, M., O'Reilly, M.G., and Taylor, C.J.L. 1990. The Forth Estuary: a nursery and overwintering area for North Sea fishes. Hydrobiologia, **195**: 89–103.

Haedrich, R.L. 1983. Estuarine fishes. *In* Estuaries and enclosed seas. *Edited by* B.H. Ketchum. Ecosystems of the world. No. 26. Elsevier, Amsterdam. pp. 183–207.

Heip, C.H.R., and Herman, P. 1995. Major biological processes in European tidal estuaries. Dev. Hydrobiol. **110**.

Henderson, P.A., and Holmes, R.H.A. 1989. Whiting migration in the Bristol Channel: a predator–prey relationship. J. Fish Biol. **34**: 409–416.

Henderson, P.A., James, D., and Holmes, R.H.A. 1992. Trophic structure within Bristol Channel: seasonality and stability in Bridgewater Bay. J. Mar. Biol. Assoc. U.K. **72**: 675–690.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia, **120**: 314–326

King, M. 1995. Fisheries biology, assessment and management. Blackwell Science Publications, Oxford, U.K.

Limburg, K. 1996. Modelling the ecological constraints on growth and movement of juvenile American shad (*Alosa sapidissima*) in the Hudson river estuary. Estuaries, **19**: 794–813.

Maes, J., Taillieu, A., Van Damme, P.A., Cottenie, K., and Ollevier, F. 1998. Seasonal patterns in the fish and crustacean community of a turbid temperate estuary (Zeeschelde Estuary, Belgium). Estuarine Coastal Shelf Sci. **47**: 143–151.

Mertz, G., and Myers, R.A. 1998. A simplified formulation for fish production. Can. J. Fish. Aquat. Sci. **55**: 478–484.

Pauly, D. 1982. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. J. Cons. Int. Explor. Mer, **39**: 175–192.

Titmus, G., Claridge, P.N., and Potter, I.C. 1978. Growth and abundance of 0-group herrings, *Clupea harengus* L., in the Severn Estuary. Zool. J. Linn. Soc. **64**: 251–260.

Van Den Broek, W.F.L. 1979. A seasonal survey of fish populations in the lower Medway Estuary, Kent, based on power station screen samples. Estuarine Coastal Mar. Sci. **9**: 1–15.

Von Bertalanffy, L. 1938. A quatitative theory of organic growth (inquiries in growth laws II). Hum. Biol. **10**: 181–213.

Wharfe, J.R., Wilson, S.R., and Dines, R.A. 1984. Observations on the fish population of an east coast estuary. Mar. Pollut. Bull. **15**: 133–136.

Bioaccumulation of trace metals in marine and estuarine amphipods: evaluation and verification of toxicokinetic models

B. Clason and G.-P. Zauke

Abstract: The suitability of the marine and estuarine gammaridean amphipods *Gammarus locustra* (Linneaus 1758), *Gammarus zaddachi* Sexton (1912), and *Gammarus salinus* Spooner (1947) from the Island of Heligoland and the Weser and Ems estuaries, northwestern Germany, as biomonitors was tested in a toxicokinetic study with the elements Cu, Zn, Pb, Cd, and Hg. The organisms responded with metal uptake upon exposure, and it was possible to estimate parameters of two-compartment or logistic regression models that were statistically different from zero. In most cases, the toxicokinetic models obtained were successfully verified in a second toxicokinetic uptake study for Cu, Zn, Pb, and Cd. Data for Cd were additionally verified with previously reported kinetic parameters. Results for Hg indicate that the organisms tested may not be suitable as biomonitors for this element. The calibration of gammarids as biomonitors was extended in a third toxicokinetic uptake study for Cu and Pb showing a linear increase from the control level to external waterborne metal exposures of 240 $\mu g\ Cu \cdot L^{-1}$ and 60 $\mu g\ Pb \cdot L^{-1}$, while Zn tended to level off above 120 $\mu g \cdot L^{-1}$ and Cd above 10 $\mu g \cdot L^{-1}$. These figures largely determine the range for which the models may serve as a predictive tool.

Résumé : Nous avons testé la possibilité d'utiliser comme bioindicateurs des amphipodes gammaridés marins et estuariens, *Gammarus locustra* Linné (1758), *Gammarus zaddachi* Sexton (1912) et *Gammarus salinus* Spooner (1947), provenant de l'île d'Helgoland et des estuaires de la Weser et de l'Ems, dans le nord-ouest de l'Allemagne, en effectuant une étude toxicocinétique portant sur les éléments Cu, Zn, Pb, Cd et Hg. Les organismes ont réagi dès l'exposition en absorbant les métaux, et il a été possible d'estimer des paramètres de modèles à deux compartiments ou de modèles de régression logistique qui étaient statistiquement différents de zéro. Dans la plupart des cas, les modèles toxicocinétiques obtenus ont été vérifiés positivement dans une deuxième étude sur l'absorption de Cu, Zn, Pb et Cd. Les données concernant le cadmium ont été de plus vérifiées à l'aide de paramètres cinétiques provenant d'une autre étude. Les résultats concernant le mercure montrent que les organismes étudiés ne conviendraient probablement pas comme bioindicateurs pour cet élément. Nous avons poursuivi l'étalonnage des gammaridés comme bioindicateurs dans une troisième étude toxicocinétique portant sur l'absorption du cuivre et du plomb, qui a montré une augmentation linéaire à partir du niveau témoin jusqu'à des expositions à des concentrations de métaux dans l'eau de 240 $\mu g \cdot L^{-1}$ pour Cu et de 60 $\mu g \cdot L^{-1}$ pour Pb, tandis que Zn atteint un plafond au-dessus de 120 $\mu g \cdot L^{-1}$ et Cd au-dessus de 10 $\mu g \cdot L^{-1}$. Ces chiffres permettent d'établir en gros la fourchette pour laquelle les modèles peuvent servir d'outils de prévision.

[Traduit par la Rédaction]

Introduction

An important precondition for using organisms as biomonitors is a net accumulation strategy (Rainbow 1995*a*; Zauke et al. 1996*b*). Investigations on the time course of uptake and clearance of metals in organisms, in relation to external metal exposures, are a first step to assess the significance of metals in aquatic systems. They provide the experimental basis for estimation of kinetic parameters of compartment models and preliminary hypotheses about underlying accumulation strategies. When metal concentrations in organisms do not readily reach a plateau phase, this indicates a net accumulation strategy under the given experimental conditions (Zauke et al. 1995). The concept of toxicokinetic studies was developed for xenobiotics in fish but has been successfully extended to metals in various aquatic invertebrates (e.g., MacLean et al. 1996; Ritterhoff et al. 1996).

In addition to a net accumulation strategy, further preconditions have to be met before organisms can be used as biomonitors. Firstly, animal collectives from different localities must show similar bioconcentration factors (BCFs) to allow a comparison between field concentrations of metals in organisms in terms of their bioavailability (Zauke et al. 1995, 1996*b*). This points to the problem of calibration of biomonitors. Verification of models involves comparison of model predictions with independent experimental data sets (Rykiel 1996; Holzbecher 1997; Bernds et al. 1998).

Gammaridean amphipods are widespread and important components of eulittoral marine, estuarine, and freshwater ecosystems and are thus interesting candidates for biomonitoring and the development of bioindicator systems (e.g., Rainbow 1995*b*; Rinderhagen et al. 2000). However,

Received September 29, 1999. Accepted March 10, 2000.
J15383

B. Clason and G.-P. Zauke.[1] Carl von Ossietzky
Universität Oldenburg, FB Biologie (ICBM),
Postfach 2503, D-26111 Oldenburg, Germany.

[1] Author to whom all correspondence should be addressed.
e-mail: gerd.p.zauke@uni-oldenburg.de

Table 1. Gammarid collectives used in toxicokinetic studies 1–3 with some information on their life history status.

						Males		Mature females		Free-living juveniles	
Collective	Species	River	km	Locality	*N*	*N* (%)	Length	*N* (%)	Length	*N* (%)	Length
G.s.-Gr	*Gammarus salinus* Spooner (1947)	Weser	56.8	Großensiel	85	45	7.8±0.5	21	5.5±0.8	34	5.0±0.2
G.z.-Te	*Gammarus zaddachi* Sexton (1912)	Ems	24.0	Terborg	96	39	10.8±0.5	40	9.0±0.2	21	6.5±0.1
G.z.-Hu	*Gammarus zaddachi* Sexton (1912)	Hunte	—	Huntebrück	67	48	9.9±0.5	18	8.1±0.4	34	6.4±0.3
G.l.-He	*Gammarus locusta* (Linnaeus 1758)	Isle of Heligoland[a]			na	—	—	—	—	—	—

Note: Length is mean body length (mm) ± 95% CIs. G.s.-Gr, toxicokinetic study 2; G.z.-Te, toxicokinetic study 1; G.z.-Hu, toxicokinetic studies 1–3 (all three collectives kept in seawater of 5‰ salinity regarding treatment and control); G.l.-He, toxicokinetic study 1 (kept in seawater of 32‰ salinity regarding treatment and control); na: no data available.

[a] German Bight, North Sea.

they are not often used in routine biomonitoring programs, in contrast with bivalves.

The objective of this study is to evaluate the suitability of gammaridean amphipods as biomonitors for trace metals and to analyze whether toxicokinetic models can be used as a predictive tool to assess environmental quality. We provide information on the toxicokinetics of Cu, Zn, Pb, Cd, and Hg in selected gammarids from the southern North Sea and the Weser and Ems estuaries (northwestern Germany). The toxicokinetic studies presented in this paper are not intended as a tool to analyze mechanisms of metal uptake in organisms; they are regarded as an integrated approach in biomonitoring, involving both field investigations and manipulative experiments (Zauke et al. 1995, 1996*b*). Thus, we did not consider the application of radioactive isotopes and applied metal mixtures instead of single-element dosing. The focus of this presentation is on bioaccumulation of waterborne metals, especially on the evaluation and verification of the kinetic parameters of two-compartment models. A current investigation (to be published elsewhere) will focus on metal uptake via food and on the relative importance of both pathways.

In total, three toxicokinetic studies were performed. In study 1, the time course of metal uptake and depuration was analyzed and model parameters were estimated to test one important precondition that must be met before organisms can be used as biomonitors. In particular, animal collectives from different localities must show similar BCFs to allow a comparison between field concentrations of metals in organisms in terms of their bioavailability (Zauke et al. 1995, 1996*b*). A verification of these model parameters was performed in a second and third toxicokinetic study, involving only uptake, in study 2 with time and in study 3 with increasing external metal dosing. The methods used for this purpose were previously outlined by Ritterhoff and Zauke (1997*a*, 1997*b*) for Arctic marine zooplankton and by Bernds et al. (1998) for polychaetes from the German Wadden Sea.

Materials and methods

Definition of terms

The term "collective" used throughout this paper denotes a gammarid sample taken at a given locality at a given time that is regarded as the basic unit of observation. By using this word, we emphasise that neither a strict random sampling of these organisms nor a definite (viz. destructive) species determination of "all specimens" collected is possible in any case (Zauke et al. 1996*a*). In toxicokinetic experiments, the time course of uptake and depuration of metals in organisms is evaluated. Here, each treatment represents one single "experimental unit" (Hurlbert 1984), whereas the independent subsamples taken at different times (see below) represent "evaluation units" (Hurlbert and White 1993). The term "uptake" denotes the incorporation of substances into the body of organisms, and "clearance" (excretion, depuration) denotes the loss of substances from the body (e.g., Rainbow et al. 1990). "Net accumulation" means that organisms are unable to match rates of excretion to rates of uptake, while "regulation" means that organisms are able to maintain constant body concentrations over a certain range of exposures. Strictly speaking, these definitions are valid only for a given set of experimental conditions, e.g., the range of external metal exposures applied and the time chosen for uptake phases, so that hypotheses based on them should be regarded only as tentative. According to OECD (1981), a "two-compartment model" involves test solutions or other exposure media as the first compartment and organisms as the second compartment. Note that in other studies, this may be termed a "one-compartment model."

Sampling and test organisms

Regarding the German North Sea coast and estuaries, only the Isle of Heligoland provides natural habitats (e.g., rocky shores covered with seaweeds) for gammaridean amphipods, enabling appropriate field sampling. In the great rivers, the animals largely colonize artificial substrates constructed to stabilize the riverbanks. During the last 20 years, the gammarid populations have dramatically decreased in these areas (which are under strong anthropogenic pressure), making it impossible to collect enough specimens in the field. Thus, we employed traps consisting of polyethylene gauze exposed to the river water at mean low water level for 2 weeks. No bait is required for this purpose because the traps accumulate great amounts of particulate organic material. One important criterion for the selection of sampling sites is easy accessibility; nevertheless, they are representative of the river systems under consideration (Zauke et al. 1988, 1995).

Four gammarid collectives sampled in September and October 1995 were considered in the toxicokinetic studies (see Table 1 for notation and details). The various gammarid collectives are representatives of different environmental conditions (viz. G.l.-He of marine, G.s.-Gr and G.z.-Te of brackish water, and G.z.-Hu of freshwater conditions) and were used to identify the ecological range within which the biomonitoring concept might be applicable. The identification of the *Gammarus* species was performed according to Lincoln (1979). Further biological characterization of the collectives involved determination of sex and body length, if enough individuals were available, which was not the case for G.l.-He. Corresponding information is summarized in Table 1.

Acclimatization

Upon arrival at the laboratory, the organisms were sorted from sand and plant debris (G.l.-He) or from the traps. To acclimate the test organisms to laboratory conditions, the four gammarid collectives were kept for at least 48 h in aquaria (water temperature about 15°C; photoperiod 14 h light : 10 h dark). The collective G.l.-He was kept in seawater of 32‰ salinity; all other collectives were kept in brackish water of about 5‰ salinity. This also applies to G.z.-Hu, which actually was collected at a freshwater site, but this species is salt tolerant and displays seasonal migrations down to the brackish water region for reproduction (Meurs and Zauke 1996). Test waters were obtained from solutions of natural sea salt (supplied for marine aquaculture, Fa. Sera) in a mixture of bidistilled and site water (80 and 20%, respectively). Salinities were checked by measuring the refractive index (Atago hand refractometer S-10, Leo Kübler GmbH, Karlsruhe); oxygen saturation was achieved by bubbling of aquaria, and the pH ranged from 7 to 8. Nitrogen components such as ammonia were not analyzed. To reduce cannibalism, the aquaria were supplied with pieces of polyethylene gauze as substrate (Borgmann et al. 1989), but cannibalism was still a problem, since 10–15% of the organisms "disappeared" during the trials, also in the controls. During acclimatization and bioaccumulation experiments, the animals were fed every second day with frozen brown seaweed (*Fucus vesiculosus*) (G.l.-He) and alder (*Alnus glutinosa*) leaves (Naylor et al. 1989). The latter were previously collected in summer, boiled for 10 min, and stored in this water in the refrigerator. This food was actually eaten by all collectives, which may be regarded as omnivorous; however, they did not distinctly increase in length and almost no moulting was observed.

Setup of the toxicokinetic studies

Toxicokinetic study 1: modelling of uptake and clearance kinetics

The experiments with G.l.-He were run at 32‰ salinity; all other experiments were run at 5‰ salinity (see previous section for details). Separate plastic buckets (6-L volume) were used employing polypropylene cages for the gammarids to facilitate water renewal (semistatic approach, every 24 h). Prior to the uptake phase, the whole system was precontaminated for 3 days to prevent absorption of metals to the walls of containers and inlets. The semistatic approach (which is in line with official test protocols, e.g., OECD 1981) was chosen for this study because of its simple and convenient setup. To study the time course of metal uptake and clearance, test organisms from the four gammarid collectives were exposed for a 10-day uptake phase to a metal mixture to assess the integrated effect of the nonessential elements Cd, Pb, and Hg and the essential elements Zn and Cu. This selection is likewise considered in German governmental monitoring programs (Anonymus 1994).

Reported soluble metal levels in ambient seawater (German Bight) are rather low: 0.035 μg Cd·L^{-1}, 0.44 μg Cu·L^{-1}, 0.15 μg Pb·L^{-1}, 1 μg Zn·L^{-1}, and < 0.0005 μg Hg·L^{-1} (Haarich and Schmidt 1993; Tappin et al. 1995). However, increasing concentrations have been reported in German water quality monitoring programs regarding the Weser and Ems estuaries (available at the time of study), yielding about 0.1–0.4 μg Cd·L^{-1}, 2–9 μg Cu·L^{-1}, 2–18 μg Pb·L^{-1}, 11–35 μg Zn·L^{-1}, and 0.07–0.61 μg Hg·L^{-1} (Anonymous 1994). The reported range demonstrates the substantial spatial and temporal variability of metals in the estuaries under consideration. In this study, we selected the nominal exposure concentrations to be 200–300 times higher than the ambient soluble metal concentrations reported for the German Bight (see above) to achieve a compromise between considering real environmental exposures (especially taking into account the more contaminated situation within the estuaries) and offering the chance to obtain a positive response in the trials (see Discussion section for more details).

We obtained the following measured soluble metal concentrations (mean ± 95% confidence intervals (CIs)) throughout the uptake phase of toxicokinetic study 1 (nominal values in parentheses): 9.9 ± 0.2 (10) μg Cd·L^{-1}, 117 ± 15 (120) μg Cu·L^{-1}, 28 ± 5 (40) μg Pb·L^{-1}, 238 ± 6 (240) μg Zn·L^{-1}, and (0.1) μg Hg·L^{-1}. However, the time course of waterborne metals in the experiments was not analyzed. We do not assume a large variability because the experimental system was precontaminated and because the ratio between the biomass introduced and the water volume was relatively large. The other experimental conditions and the measures of water quality were the same as in the acclimatization phase mentioned above.

Parallel to the uptake experiments, control gammarids were kept under conditions similar to those of the acclimatization phase (viz. G.l.-He at 32‰ salinity; all other collectives at 5‰ salinity). During the clearance phase, the gammarid collectives from toxicokinetic study 1 were kept separately within polypropylene cages under control conditions for another 10 days.

The number of animals added to each treatment was about 450. The overall mortality in this trial was 10–15% in the controls and 20–25% in the metal treatments, including loss due to cannibalism. Gammarids from each collective (exposure and control) were randomly sampled on days 0, 0.5, 1, 2, 3, 4, 6, 8, and 10. During the clearance phase, samples were taken on days 11, 13, 15, 17, 19, and 20, if there were still animals left. The gammarids were briefly rinsed with double-distilled water and surface water was removed from the animals by drying on good-quality filter paper. Subsequently, the animals taken from each collective were allocated to three Eppendorf reaction tubes (polypropylene, 1.5-mL volume). Thus, we obtained three independent replicates (evaluation units) per gammarid collective and sampling day consisting of three to five specimens, respectively, for the treatments as well as for the controls. All gammarid samples for metal determination were stored frozen at −20°C.

Water collections for metal analyses of treatments (100 mL) were done between two water exchanges (to reflect a mean exposure) with the aid of an acid-rinsed pipette. The controls were not sampled because at the time of the experiments, no facilities were available to detect very low metal concentrations in salt water. The samples were stored at −20°C in precleaned polyethylene containers until we had the opportunity to analyze them with the aid of polarography (see below).

Toxicokinetic study 2: verification of toxicokinetic model parameters

An independent toxicokinetic study (study 2) was set up to achieve a first verification of model parameters obtained from study 1. This time, only the collectives G.z.-Hu and G.s.-Gr were considered in the trials. Furthermore, the experiments consisted only of an uptake phase of 10 days. With these exceptions, the experimental details, exposure concentrations of the metal mixtures, sampling scheme of tissue and water collections, number of animals added to the treatments, and mean survival of the test organisms were largely the same as reported above for study 1.

Toxicokinetic study 3: uptake, verification of toxicokinetic model parameters

To allow a second verification of toxicokinetic models, semistatic uptake experiments covering a wider exposure range were set up (exposure time 4 days, gammarid collective G.z.-Hu used). Details of exposure conditions are given in Table 2. The range selected involved the concentrations of toxicokinetic studies 1 and 2, in addition, one lower level and two higher levels (see Discussion section for more details). Polyethylene buckets filled with 2 L of test water were used as experimental vessels. The experiment started with 30 specimens for each treatment. Again, test solutions were renewed every day. Dead organisms were recorded and removed every day, while the organisms surviving were collected by the end of the experiment, leading to four subsamples (evaluation units) from two independent replicates. Further sample preparation was the same as outlined for toxicokinetic studies 1 and 2. In contrast with these studies, animals were not fed in study 3, leading to a higher mortality (25–40% on day 4), eventually due to increased cannibalism.

Analytical procedures

Gammarid samples were first freeze-dried and then homogenized by using a small boron carbide mortar and pestle to avoid losses of biomass. Aliquots of about 10 mg of dried material (representing three to five gammarids, respectively) were digested for 1 h at 96°C with 50 µL of HNO_3 (70–71%, Baker Instra-Analysed) in 1.5-mL Eppendorf reaction tubes (Zauke et al. 1996*a*). The digests were made up to a volume of 1 mL with bidistilled water. After appropriate dilution, the final sample and standard solutions were adjusted to concentrations of 1.75% HNO_3. Cadmium, Cu, and Pb were assayed using sequential multielement graphite tube atomic absorption spectroscopy (Varian Techtron, AA-975, GTA-95) with platform atomization. Zinc was assayed using an air–acetylene flame and a manual microinjection method (100-µL sample volume). For all determinations, deuterium background correction was applied.

To determine Hg, aliquots of about 10 mg of dried material were digested for 1 h at 96°C with 50 µL of HNO_3 (70–71%, Baker Instra-Analysed) and 50 µL of HCl (37%, Riedel de Haen, p.a.) in 1.5-mL Eppendorf reaction tubes (safe twist) (Ritterhoff and Zauke 1997*c*). Mercury determinations involved reduction of the digest with $SnCl_2$ (10 g per 100 mL of solution of 3.7% HCl), evaporation of Hg by application of nitrogen (grade 5.0) for 3 min, and subsequent amalgamation on a Pt/Au net (Varian Techtron, MK II, AA 975).

Quality assurance was performed in line with German GLP regulations (Anonymous 1999) using the following documented criteria: stability of instrumental recalibration, precision of parallel injections (normally showing a coefficient of variation of 1–5%), and analytical blanks (also reflecting the digestion procedure). Furthermore, the precision and validity were evaluated using three certified reference materials that were randomly allocated within the determinations (see Table 3). The analyzed values for the reference materials are largely in good agreement with the certified values. Limits of detection were calculated according to Büttner et al. (1980) as mean blank plus 2.6 SD of a "low value" and according to DIN-32645 (1994). One advantage of the first method is that it relies on real samples near the blanks, while the second method relies on the calibration line, assuming linearity which is not given in any case. The different results are displayed in Table 3.

Water samples from the exposure treatments were analyzed in standard addition mode by polarography (Metrohm 746 VA trace analyser, 747 Stand) using a hanging mercury drop electrode and a reference electrode filled with 3 M KCl. Prior to analyses, samples were degassed for 290 s with nitrogen (grade 5.0). Peaks were detected under the following conditions: –970 mV (Zn), –600 mV (Cd), –390 mV (Pb), and –160 mV (Cu), with application of differential pulse voltametry for Zn and differential pulse – anodic stripping voltametry for the other elements, and acidification of samples with HNO_3 (Baker Instra-Analysed) to pH 2. The linearity of the additions was checked by linear regression analysis leading to adjusted R^2 values of ≥0.999.

Table 2. Exposure concentrations ($\mu g \cdot L^{-1}$) for the uptake studies using metal mixtures.

	Cd		Pb		Zn		Cu	Hg
Treatment	*m*	*n*	*m*	*n*	*m*	*n*	*n*	*n*
1	5.4/5.5	5	10/16	20	110/115	120	60	0.05
2	10/10	10	21/34	40	241/241	240	120	0.10
3	13/14	15	39/42	60	365/368	360	180	0.15
4	17/17	20	48/51	80	480/502	480	240	0.20

Note: *m*, duplicate measurements; *n*, nominal values. Metal concentrations in the control were not analyzed.

Table 3. Quality assurance using certified reference materials randomly allocated within the determinations.

	NIST SRM 1566 (oyster tissue)			CRM 278 (mussel tissue)		
	Analyzed	*N*	Certified	Analyzed	*N*	Certified
Cd	3.2±0.2	14	3.5±0.4	0.31±0.02	14	0.34±0.02
Pb	0.4±0.2	12	0.48±0.04	1.8±0.5	16	1.91±0.04
Hg	—	—	—	0.17±0.01	10	0.19±0.01
Cu	66±4	11	63.0±3.5	11±2	16	9.6±0.16
Zn	949±157	8	852±14	72±4	14	76±2

Note: Values are means ± 95% CIs ($\mu g \cdot g$ dry $weight^{-1}$). *N*, numbers of independent determinations. Hg in NRC Dorm-1 (dogfish muscle): 0.76 ± 0.03 $\mu g \cdot g$ dry $weight^{-1}$ ($N = 11$, analyzed) versus 0.784 ± 0.034 $\mu g \cdot g$ dry $weight^{-1}$ (certified); limits of detection for biological material: Cd = 0.1 $\mu g \cdot g$ dry $weight^{-1}$, Pb = 0.6 $\mu g \cdot g$ dry $weight^{-1}$, Hg = 0.03 $\mu g \cdot g$ dry $weight^{-1}$, Cu = 9.4 $\mu g \cdot g$ dry $weight^{-1}$, and Zn = 15 $\mu g \cdot g$ dry $weight^{-1}$ according to Büttner et al. (1980) (2.6 SD of a "low value") and Cd = 0.2 $\mu g \cdot g$ dry $weight^{-1}$, Pb = 0.2 $\mu g \cdot g$ dry $weight^{-1}$, Hg = 0.02 $\mu g \cdot g$ dry $weight^{-1}$, Cu = 4.2 $\mu g \cdot g$ dry $weight^{-1}$, and Zn = 3.6 $\mu g \cdot g$ dry $weight^{-1}$ according to DIN-32645 (1994); limits of detection for water samples: Cd = 0.05 $\mu g \cdot L^{-1}$, Pb = 0.4 $\mu g \cdot L^{-1}$, Hg = 0.15 $\mu g \cdot L^{-1}$, Cu = 0.4 $\mu g \cdot L^{-1}$, and Zn = 2 $\mu g \cdot L^{-1}$ according to Büttner et al. (1980).

Data evaluation

Regarding toxicokinetic study 1, the time courses of metal uptake and depuration were evaluated employing two-compartment models with test solutions as the first compartment and animals as the second compartment. (Zauke et al. 1995; Ritterhoff et al. 1996). Model parameters were estimated simultaneously from the following equations using nonlinear iterative least square methods. For the uptake phase ($0 < t < t^*$ where t^* is the end of the uptake phase):

$$(1) \qquad C_A = C_0 + C_W \frac{k_1}{k_2}(1 - e^{-k_2 t})$$

For the clearance phase ($t > t^*$):

$$(2) \qquad C_A = C_0 + C_W \frac{k_1}{k_2}(e^{-k_2(t-t^*)} - e^{-k_2 t})$$

where C_A is mean metal concentration in animals (micrograms per gram dry weight), C_0 is a constant ($t = 0$), C_W is the mean measured metal exposure (milligrams per litre), k_1 is the rate constant for the uptake phase (per day), and k_2 the rate constant for the clearance phase (per day).

The calculations were done with SYSTAT for Windows (version 8.0) using the NONLIN subroutine and piecewise regression option (Wilkinson 1998, p. 643 ff.). The goodness-of-fit was assessed with the aid of Lilliefors probabilities (two-tailed) to test the normal distribution of residuals (Wilkinson 1998, p. 711), a corrected R^2 (approximate coefficient of determination), and approximate *t* ratios (parameter estimates/approximate SEs, only for compartment models; Bates and Watts 1988, p. 90). BCFs for theoretical equilibrium were calculated as

Table 4. Toxicokinetics of metals in different gammarid collectives: results of two-compartment models obtained from toxicokinetic study 1 using eqs. 1 and 2.

Metal	Collective	C_0	C_{con} ± 95% CI	k_1 ± 95% CI	t	k_2 ± 95% CI	t	BCF	LIP	df	R^2
Zn	G.l.-He	48	51±12	61±17	7.4	0.17±0.06	5.8	358	0.070	50	0.617
	G.z.-Hu	70	72±18	39±14	5.9	0.14±0.06	4.8	279	0.400	41	0.561
	G.z.-Te	—	79±10	—	—	—	—	—	—	—	—
Cu	G.l.-He	64	64±11	149±32	9.4	0.10±0.03	7.0	1490	0.134	53	0.730
	G.z.-Hu	95	111±15	168±39	8.7	0.10±0.03	7.0	1680	0.107	50	0.689
	G.z.-Te	99	83±10	170±43	8.0	0.10±0.04	5.4	1700	0.426	40	0.799
Pb	G.l.-He	3.6	2.0±1.0	98±29	6.9	0.11±0.04	5.2	890	0.238	55	0.569
	G.z.-Hu	2.2	3.5±1.4	146±47	6.3	0.15±0.06	5.3	973	0.057	44	0.582
	G.z.-Te	3.5	2.8±0.9	96±30	6.5	0.08±0.04	3.7	1200	0.039	40	0.745

Note: See Table 1 for notation and further details. C_0, location parameter of two-compartment model (µg·g dry weight^{-1}) (t = 0); C_{con}, metal concentrations in gammarids from control experiments (µg·g dry weight^{-1}); k_1, rate constant for uptake phases (per day); k_2, rate constant for clearance phases (per day); t, approximate t ratio of corresponding parameters (= estimate/SE); critical t value (two-sided), $t_{40;0.05} = 2.02$; BCF, bioconcentration factor = k_1/k_2 (dry weight basis) (eq. 3); LIP, Lillifors probability (two-tailed) test of normality of residuals ($\alpha = 0.01$); R^2, approximate coefficient of determination.

Table 5. Toxicokinetics of metals in different gammarid collectives: results of logistic regression models obtained from toxicokinetic study 1 using eq. 4.

Metal	Collective	C_{con} ± 95% CI	C_{eq} ± 95% CI	t	A ± 95% CI	t	B ± 95% CI	t	BCF	LIP	df	R^2
Cd	G.l.-He	0.12±0.03	1.8±0.1	36	3.2±1.1	5.6	–0.69±0.27	–5.1	183	0.001	54	0.881
	G.z.-Hu	0.14±0.03	4.1±0.4	20	2.6±1.3	3.9	–0.66±0.38	–3.6	416	0.209	44	0.770
	G.z.-Te	0.14±0.03	4.9±0.4	25	2.5±0.6	7.9	–0.52±0.16	–6.6	494	0.647	43	0.907
Hg	G.l.-He	0.09±0.02	0.12±0.01	21	5.3±3.8	2.7	–0.97±0.72	–2.7	1240	0.452	56	0.784
	G.z.-Hu	0.09±0.02	0.12±0.02	15	5	—	–0.73±0.14	–10	1190	0.006	47	0.720
	G.z.-Te	0.12±0.03	0.20±0.03	14	3.3±1.2	5.4	–0.51±0.22	–4.7	2000	0.401	41	0.847

Note: Notation as in Tables 1 and 4. C_{eq}, A, B, model parameters of logistic regression; critical t value (two-sided), $t_{40;\,0.05} = 2.02$.

$$\text{(3)} \qquad \mathrm{BCF} = \frac{k_1}{k_2} = \lim \frac{C_A}{C_W} (\text{for } t \rightarrow \infty).$$

For some experimental data, best approximation was achieved using a logistic regression model:

$$\text{(4)} \qquad C_A = C_{con}\frac{C_{eq}}{(1 + e^{(a+bt)})} = C_{con}\frac{C_W \mathrm{BCF}}{(1 + e^{(a+bt)})}$$

where a, b, and C_{eq} are model constants and C_{con} is the measured concentration of metals in the control organisms (micrograms per gram dry weight).

Regarding toxicokinetic study 2, model predictions for each of the experimental units and metal exposure treatments given in Table 2 were calculated using either eq. 1 or eq. 4 with C_W being constant and the time being used as the independent variable. Values for k_1, k_2, or parameters of the logistic regression (were appropriate) were taken from results of toxicokinetic study 1 (Tables 4 and 5). The same applies to toxicokinetic study 3, with the only exception being that in this case, the time is constant (4 days) and C_W is taken as the independent variable (measured values m in Table 2). As can be seen from eqs. 1 and 4, C_A is a linear function of C_W, with all other parameters being constant within a given experiment. Consequently, model predictions for each of the experimental units analyzed in this study are described by a straight line.

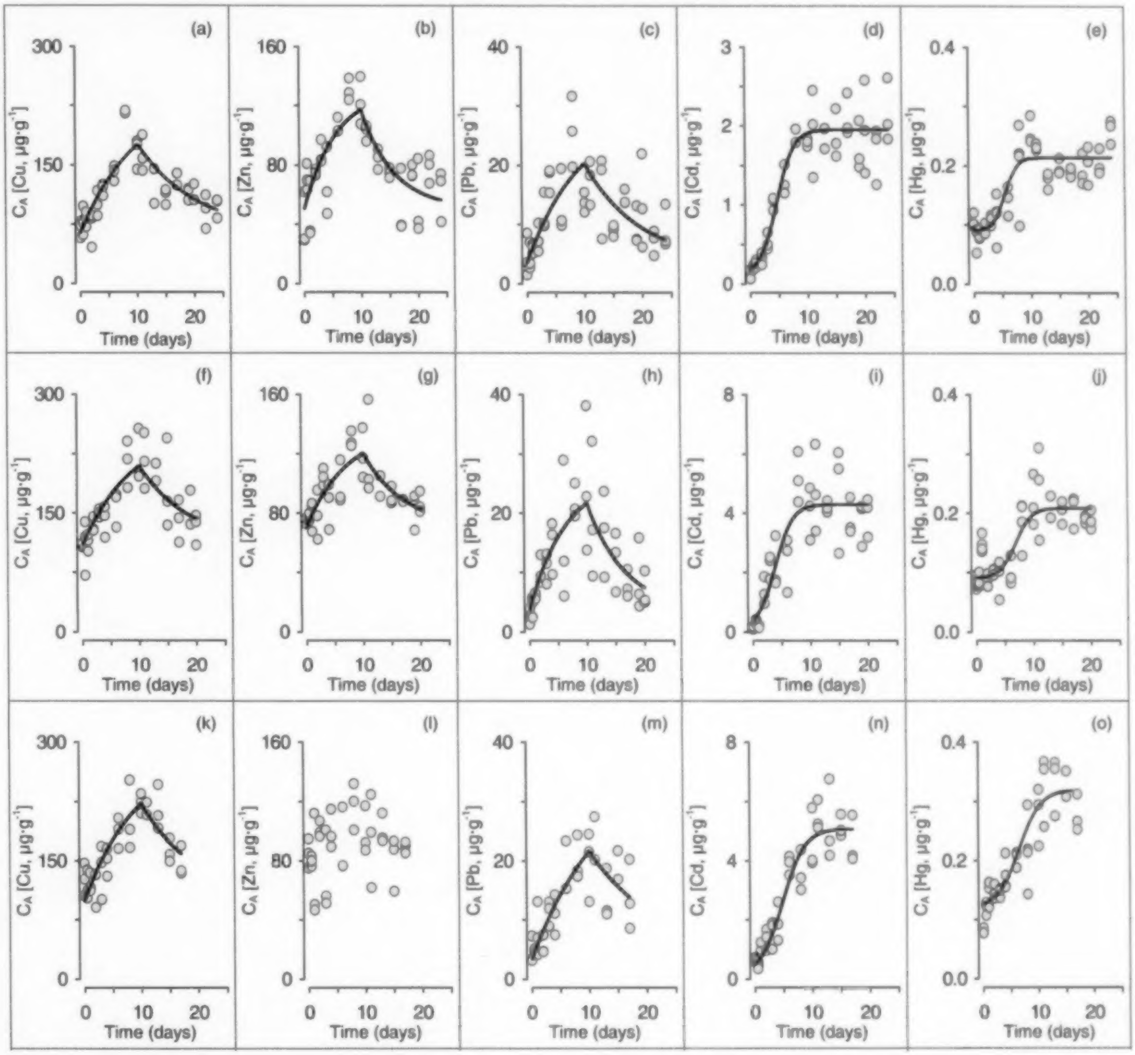
Linear regression analysis using SYSTAT 8.0. was employed to assess the goodness-of-fit between observed data and model predictions, assuming that the slope yields a value of 1 in the case of complete agreement. If the 95% CI includes the value 1, this is regarded as an indication of fairly good agreement between the corresponding data sets. A similar comparison was made for Cd using various toxicokinetic parameters previously reported in the literature (for details, see Zauke et al. 1995; Ritterhoff et al. 1996; Rinderhagen et al. 2000).

Results

Results of toxicokinetic study 1 are shown in Fig. 1; the estimated parameters of the two-compartment and logistic regression models appear in Tables 4 and 5. The organisms respond with distinct metal uptake upon external metal exposure with the only exception of Zn in collective G.z.-Te, where only a slight change of internal metal levels is obvious (Fig. 1*l*). The uptake of Cu, Zn, and Pb does not reach a plateau within the uptake phase. The gammarids eliminate accumulated Cu, Zn, and Pb significantly during the clearance phase. This is not the case for Cd and Hg, where due to a lack of metal elimination in the clearance phase, logistic regression models were employed.

Statistical information (Tables 4 and 5) and inspection of Fig. 1 suggest that our estimated models give a fair description of the observed data. Sufficiently good fits of compartment models were obtained for Cu and Pb regarding all collectives, while for Zn, this was only possible for G.l.-He and G.z.-Hu. Logistic regression analysis was successfully employed for Cd and Hg, with the advantage that BCFs can be calculated even when compartment models are not applicable. Regarding approximate t ratios of the compartment

Fig. 1. Uptake and clearance of (*a, f, k*) Cu, (*b, g, l*) Zn, (*c, h, m*) Pb, (*d, i, n*) Cd, and (*e, j, o*) Hg in gammarid collectives (*a–e*) G.l.-He, (*f–j*) G.z.-Hu, and (*k–o*) G.z.-Te (notation in Table 1) from toxicokinetic study 1. Waterborne metal exposures: 117 µg $Cu{\cdot}L^{-1}$, 238 µg $Zn{\cdot}L^{-1}$, 28 µg $Pb{\cdot}L^{-1}$, 9.9 µg $Cd{\cdot}L^{-1}$, and 0.1 µg $Hg{\cdot}L^{-1}$. Comparison of observed values (circles) with predictions of two-compartment models (Cu, Zn, Pb; line, using eqs. 1 and 2; kinetic data in Table 4) and logistic regression models (Cd, Hg; line, using eq. 4; parameters in Table 5). Day 10: end of uptake phases; see text for more details.

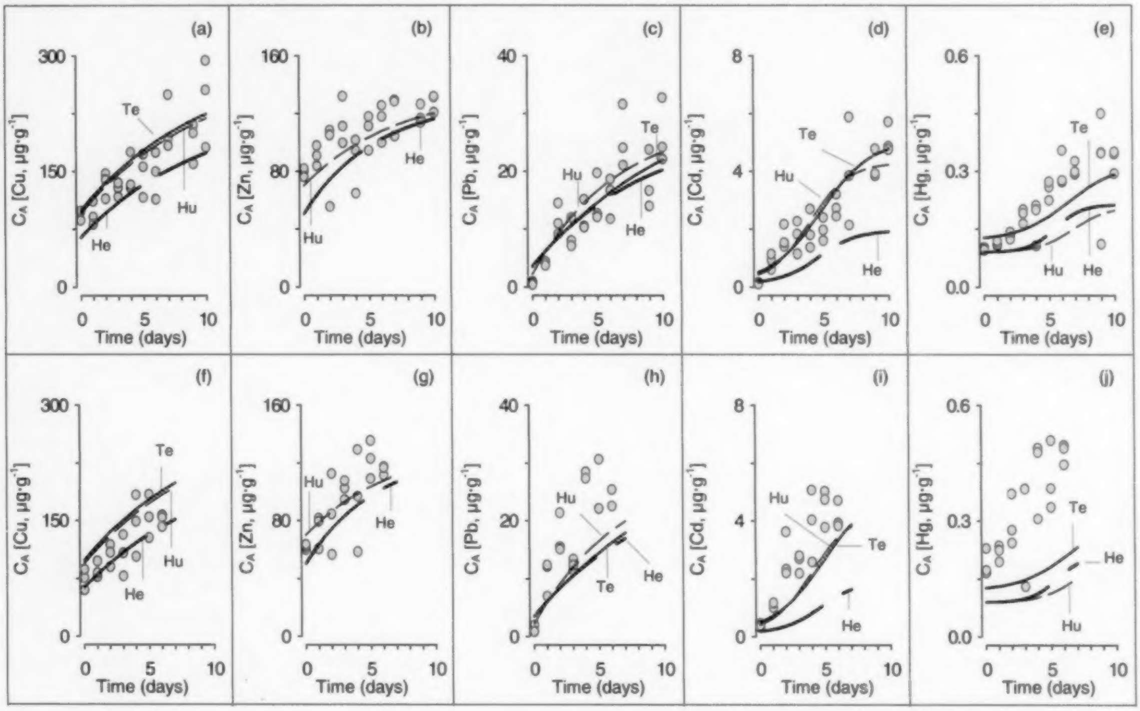


models, all parameters are significantly different from zero. For both types of models, the corrected R^2 values indicate that a high proportion of the variance can be explained by the models. Lillifors probabilities support the hypothesis of normality of the residuals ($\alpha = 0.01$) for most regressions. Thus, important preconditions for employing these methods are met. This is further supported by the fact that there is largely good agreement between the measured control levels C_{con} and the model constants C_0.

Results obtained for toxicokinetic study 2 are presented in Fig. 2, showing a comparison of observed Cu, Zn, Pb, Cd, and Hg net uptake in the gammarid collectives G.z.-Hu and G.s.–Gr with model predictions using eqs. 1 and 4. As outlined previously, values for k_1, k_2, and parameters of the logistic regression models were taken from the results of toxicokinetic study 1 (Tables 4 and 5) (see Discussion section for more details).

Results obtained for toxicokinetic study 3 with increased dosing of metal mixtures outlined in Table 2 are presented in Fig. 3, showing a comparison of observed Cu, Zn, Pb, Cd, and Hg net uptake in the gammarid collective G.z.-Hu with model predictions using eqs. 1 and 4. Again, values for k_1, k_2, and parameters of the logistic regression models were taken from the results of toxicokinetic study 1 (Tables 4 and 5), while values for C_W and t are given by the conditions of this independent uptake experiment (see Table 2).

Fig. 2. Verification of toxicokinetic model parameters obtained in toxicokinetic study 1 (Fig. 1; Tables 4 and 5) with independent experiments (toxicokinetic study 2, only uptake phase). Comparison of observed (*a*, *f*) Cu, (*b*, *g*) Zn, (*c*, *h*) Pb, (*d*, *i*) Cd, and (*e*, *j*) Hg net uptake (circles) in different gammarid collectives (*a*–*e*) G.z.-Hu and (*f*–*j*) G.s.-Gr (notation in Table 1) with model predictions using (*a*–*c*, *f*–*h*) eq. 1 and (*d* and *e*, *i* and *j*) eq. 4. Values for k_1, k_2, and parameters of the logistic regression models were taken from toxicokinetic study 1 (Tables 4 and 5). Displayed are models for G.z.-Te (solid line), G.l.-He (long-dashed line), and G.z.-Hu (short-dashed line); see text for more details.



The results of assessment of the goodness-of-fit between observed data and model predictions, including data for slopes, *t* values, 95% CIs of slopes, and Lillifors probabilities are presented in Tables 6 and 7 for all comparisons given in Figs. 2 and 3.

Discussion

Toxicokinetic study 1: calibration of biomonitors

Strictly speaking, a comparison of BCFs is only possible on a heuristic basis, since statistical techniques to test, for example, the null hypothesis of equality of BCFs are not at hand, due to theoretical considerations (for details, see Ritterhoff et al. 1996). Nevertheless, our results suggest that the calculated BCFs for each element are largely in good agreement comparing the different gammarid collectives. This may be regarded as a first successful calibration of the gammarid collectives investigated. The only exception is noted for Cd in G.l.-He. Since these gammarids were kept in seawater of 32‰ salinity during the acclimatization and uptake experiments, this most probably indicates a salinity effect. Various reports exist about the influence of salinity on the accumulation of metals, especially for Cd (e.g., Bjerregaard and Depledge 1994; Rainbow 1995*b*; Wright 1995), indicating that the bioavailability of Cd is affected by salinity due to a decrease in available free Cd ions with increasing salinity (Rainbow 1995*b*).

The BCFs obtained in our experiments are largely within the range reported in the literature. Previous studies on *Gammarus zaddachi* and *Gammarus salinus* showed kinetic BCFs for Cd in the range of 400–1200, with waterborne Cd exposures ranging from 17 to 52 µg Cd·L^{-1} (Zauke et al. 1995; Ritterhoff et al. 1996). Further information is available for the Arctic marine amphipod *Themisto abyssorum*, where reported BCFs for Pb (1800), Cu (1400), and Zn (190) agree rather well with our data (Ritterhoff and Zauke 1997*b*). Kinetic BCFs are also available for the freshwater amphipod *Hyalella azteca* yielding 1900 for Pb (waterborne exposure 100 µg Pb·L^{-1}; MacLean et al. 1996) and 4600 for Cu and 5100 for Zn (waterborne exposure 50 µg Cu·L^{-1} and 300 µg Zn·L^{-1}; Borgmann and Norwood 1995).

Toxicokinetic studies 2 and 3: verification of model parameters

A comparison of observed metal concentrations in gammarids obtained in toxicokinetic studies 2 and 3 with model predictions obtained from toxicokinetic study 1 is presented in Figs. 2 and 3. The emphasis of this approach is to extend the calibration of gammarids as biomonitors (see previous section) and to demonstrate the potential of com-

Fig. 3. Verification of toxicokinetic model parameters obtained in toxicokinetic study 1 (Fig. 1; Tables 4 and 5) with an independent uptake experiment (toxicokinetic study 3, uptake phase 4 days, different exposures given in Table 2). Comparison of observed (*a*) Cu, (*b*) Zn, (*c*) Pb, (*d*) Cd, and (*e*) Hg net uptake (circles) in the gammarid collective G.z.-Hu (notation in Table 1) with model predictions using (*a–c*) eq. 1 and (*d–e*) eq. 4. Values for k_1, k_2, and parameters of the logistic regression models were taken from toxicokinetic study 1 (Tables 4 and 5), while values for C_W and t are given by the conditions of the independent uptake experiment (Table 2). Displayed are models obtained for G.z.-Te (solid line), G.l.-He (long-dashed line), and G.z.-Hu (short-dashed line); see text for more details.

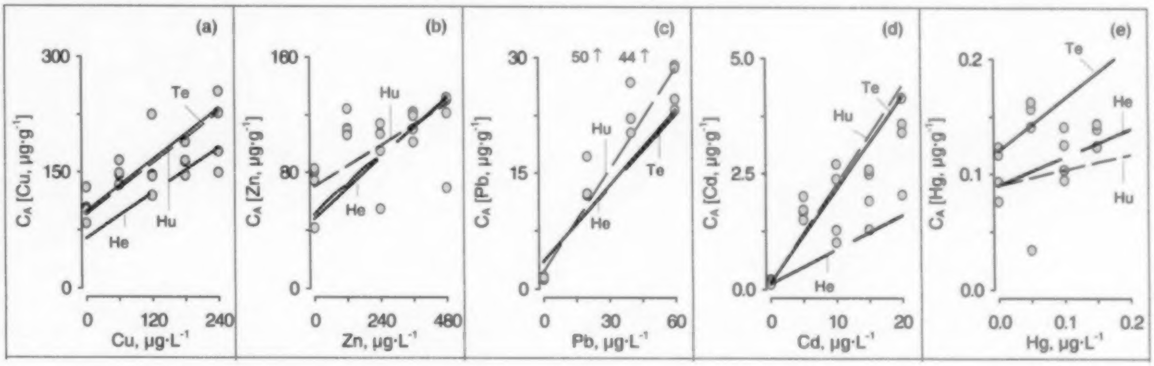


Table 6. Linear regression of observed metal concentrations in gammarids (toxicokinetic study 2) versus different model predictions obtained from toxicokinetic study 1 (see Fig. 2).

Metal	Observed	Model prediction	Slope	*t* value	95% CIs	LIP
Cu	G.z.-Hu	G.l.-He	1.20	7.4	0.87–1.54	0.581
		G.z.-Hu	1.07	7.4	0.77–1.36	0.580
		G.z.-Te	1.06	7.4	0.77–1.35	0.580
Zn	G.z.-Hu	G.l.-He	0.63	5.1	0.38–0.88	0.114
		G.z.-Hu	0.88	5.2	0.53–1.23	0.197
Pb	G.z.-Hu	G.l.-He	1.39	9.1	1.08–1.70	0.108
		G.z.-Hu	1.09	9.0	0.84–1.34	0.071
		G.z.-Te	1.25	9.1	0.97–1.53	0.172
Cd	G.z.-Hu	G.l.-He	2.21	9.7	1.75–2.68	0.496
		G.z.-Hu	1.00	9.1	0.77–1.22	0.478
		G.z.-Te	0.95	10.4	0.76–1.14	0.673
Hg	G.z.-Hu	G.l.-He	1.71	8.3	1.29–2.13	0.047
		G.z.-Hu	1.95	6.7	1.36–2.54	0.310
		G.z.-Te	1.39	7.7	1.03–1.76	0.011
Cu	G.s.-Gr	G.l.-He	1.12	6.3	0.74–1.49	0.664
		G.z.-Hu	1.00	6.3	0.66–1.32	0.662
		G.z.-Te	0.98	6.3	0.65–1.31	0.664
Zn	G.s.-Gr	G.l.-He	1.05	5.2	0.62–1.47	0.056
		G.z.-Hu	1.52	5.2	0.91–2.14	0.067
Pb	G.s.-Gr	G.l.-He	2.02	7.2	1.42–2.62	0.063
		G.z.-Hu	1.51	7.3	1.07–1.94	0.200
		G.z.-Te	1.90	7.0	1.32–2.47	0.031
Cd	G.s.-Gr	G.l.-He	2.95	6.7	2.02–3.87	0.131
		G.z.-Hu	1.26	7.6	0.91–1.60	0.175
		G.z.-Te	1.35	7.2	0.95–1.74	0.035
Hg	G.s.-Gr	G.l.-He	3.28	4.7	1.82–4.74	0.012
		G.z.-Hu	6.37	4.7	3.54–9.21	0.003
		G.z.-Te	3.42	5.5	2.11–4.74	0.171

Note: Notation as in Tables 1 and 4. Regression constants statistically not different from zero; critical *t* values (two-sided), $t_{20;0.05} = 2.09$ (applies to observed G.s.-Gr) and $t_{30;0.05} = 2.04$ (applies to observed G.z.-Hu).

Table 7. Linear regression of observed metal concentrations in gammarids (toxicokinetic study 3) versus different model predictions obtained from toxicokinetic study 1 (see Fig. 3).

Metal	Observed	Model prediction	Slope	t value	df	Critical t value	95% CIs	LIP
Cu	G.z.-Hu	G.l.-He	0.70	3.9	18	2.10	0.32–1.08	0.262
		G.z.-Hu	0.62	3.9	18	2.10	0.29–0.96	0.260
		G.z.-Te	0.62	3.9	18	2.10	0.28–0.95	0.262
Zn	G.z.-Hu	G.l.-He	na	—	—	—	—	na
		G.z.-Hu	na	—	—	—	—	na
Pb	G.z.-Hu	G.l.-He	1.32	11.0	13	2.16	1.06–1.58	0.102
		G.z.-Hu	0.96	11.0	13	2.16	0.77–1.14	0.102
		G.z.-Te	1.28	11.0	13	2.16	1.02–1.53	0.101
Cd	G.z.-Hu	G.l.-He	1.79	6.2	19	2.09	1.19–2.40	0.763
		G.z.-Hu	0.62	6.2	19	2.09	0.41–0.83	0.776
		G.z.-Te	0.66	6.2	19	2.09	0.43–0.88	0.763
Hg	G.z.-Hu	G.l.-He	ns	1.2	15	2.13	—	—
		G.z.-Hu	ns	1.2	15	2.13	—	—
		G.z.-Te	ns	1.2	15	2.13	—	—

Note: Notation as in Tables 1 and 4. Regression constants statistically not different from zero; critical t values two-sided, $\alpha = 0.05$; na, not applicable (due to patterns in the residual and autocorrelation plots of the SYSTAT output); ns, statistically not different from zero.

partment models as a predictive tool. The results presented in Fig. 2*a*, for example, were obtained by calculating model predictions with eq. 1, taking the values of the parameters for each collective from Table 4: $\mathrm{Cu} = 64 + 0.117 \times 149/0.1(1 - (\exp(-0.1 \times t_i)))$ in the case of G.l.-He, $\mathrm{Cu} = 110 + 0.117 \times 142/0.11(1 - (\exp(-0.11 \times t_i)))$ in the case of G.z.-Hu, etc. The same applies to Fig. 3 with the only exception that in this case, C_W is the independent variable instead of t, e.g., leading to $\mathrm{Cu} = 99 + C_{W_i} \times 170/0.1(1-(\exp(-0.1 \times 4)))$ for G.z.-Te.

The goodness-of-fit between observed data and model predictions can be assessed from the information compiled in Tables 6 and 7. Regarding toxicokinetic study 2 (Table 6), fairly good agreement can be inferred for Cu, Zn, Pb, and Cd (collective tested: G.z.-Hu) as well as for Cu, Zn, and Cd (collective tested: G.s.-Gr), where the 95% CIs of the slopes largely include the value 1. Only the model G.l.-He clearly underestimates the observed data. As mentioned previously, these gammarids were kept in seawater of 32‰ salinity during the acclimatization and uptake experiments, most probably indicating a salinity effect (see previous section). More striking is the failure to predict Pb for collective G.s.-Gr in contrast with G.z.-Hu. In both cases, experiments were performed in seawater of 5‰ salinity. A possible explanation might be that collective G.s.-Gr showed the smallest body length at the time of sampling. Accumulated metals may be influenced by life history status (Bäumer et al. 1991) or by different surface areas of the body (which are related to the length), as has been demonstrated for organic chemicals in marine algae (Wang et al. 1997). The results obtained for Hg are not promising at all, eventually indicating that the gammarids tested are not suitable as biomonitors for this element.

Regarding toxicokinetic study 3 (Table 7), fairly good agreement can be inferred for Cu and Pb. This is eventually also true for Zn, but in this case, a statistical analysis was not applicable due to patterns in the residual and autocorrelation plots (not shown here). In the case of Cd, we do not find good correspondence between observed and predicted values: again, the model G.l.-He underestimates the observations (as mentioned above), while the other models fail because Cd apparently does not show a linear increase with increasing exposure. Obviously, the range of external metal exposures to which the models are applicable is limited (see next section for further details). An almost linear relationship between accumulated Cu and waterborne exposure was reported for *H. azteca* within a range of approximately 25–100 μg $\mathrm{Cu \cdot L^{-1}}$ (Borgmann and Norwood 1995), with values distinctly levelling off above 150 μg $\mathrm{Cu \cdot L^{-1}}$. Likewise, a log–log linear relationship was demonstrated for Pb in *H. azteca* within a range of approximately 10–100 μg $\mathrm{Pb \cdot L^{-1}}$ (MacLean et al. 1996).

A comparison of observed Cd concentrations in gammarids (toxicokinetic studies 1 and 2) with model predictions obtained from previous toxicokinetic studies reported in the literature (Zauke et al. 1995; Ritterhoff et al. 1996) is presented in Table 8. Although in the present paper, it was not possible to fit a two-compartment model to Cd in gammarids, we find much remarkably good agreement between observed and predicted values in Table 8, showing that not a unique set of k_1 and k_2 values exists that might be suitable as a prognostic tool, but rather a whole family of appropriate k_1 and k_2 combinations (Rinderhagen et al. 2000).

Inferences about accumulation strategies

An important precondition for using organisms as biomonitors is a net accumulation strategy (Zauke et al. 1996*b*). In our study, this condition is met to a great extent, since a plateau phase is not readily reached in toxicokinetic studies 1 and 2, at least under the constraints of the exposure levels applied and the time chosen for uptake phases. However, the results for Cd and Hg are not unequivocal due to a lack of depuration within approximately 10 days. Further support is provided by the results of toxicokinetic study 3, where Cu and Pb in the gammarids increase with increased external waterborne metal exposure. For Zn, Cd, and Hg, such an increase is less distinct; the data may show an increase, but they are too variable to show it significantly.

In the case of Zn, this finding eventually indicates a ten-

Table 8. Linear regression of observed Cd concentrations in gammarids (toxicokinetic studies 1 and 2) versus different model predictions obtained from previous toxicokinetic studies reported in the literature.

Study	Observed	Model prediction	Reference	Reported k_1	Reported k_2	Slope	t value	95% CIs	LIP
1	G.l.-He	J1	Z	25.9	0.061	1.00	20.1	0.87–1.07	1.000
		Q1	Z	24.9	0.066	1.03	20.0	0.92–1.14	1.000
1	G.z.-Hu	Ga	R	61.4	0.132	1.35	10.0	1.07–1.63	0.552
		Hu	R	73.2	0.127	1.11	10.1	0.88–1.33	0.601
		K	R	103.0	0.253	1.18	9.2	0.92–1.45	0.270
		P1	Z	65.8	0.121	1.20	10.0	0.96–1.45	0.686
		J2	Z	52.8	0.056	1.15	10.0	0.91–1.39	0.050
		Q2	Z	70.6	0.060	0.87	10.0	0.69–1.05	0.072
1	G.z.-Te	Ga	R	61.4	0.132	1.17	11.7	0.96–1.38	0.745
		Hu	R	73.2	0.127	0.96	11.8	0.79–1.13	0.629
		K	R	103.0	0.253	1.01	9.6	0.80–1.23	0.555
		P1	Z	65.8	0.121	1.05	11.9	0.87–1.23	0.577
		J2	Z	52.8	0.056	1.01	12.6	0.84–1.17	0.225
2	G.z.-Hu	Ga	R	61.4	0.132	1.30	9.8	1.03–1.58	0.279
		Hu	R	73.2	0.127	1.07	9.9	0.85–1.29	0.205
		K	R	103.0	0.253	1.14	8.2	0.85–1.42	0.492
		J2	Z	52.8	0.056	1.12	10.8	0.91–1.33	0.115
2	G.s.-Gr	Hu	R	73.2	0.127	1.37	9.5	1.07–1.67	0.081
		K	R	103.0	0.253	1.33	9.7	1.04–1.61	0.446
		Q2	Z	70.6	0.060	1.17	9.1	0.90–1.44	0.043
		P2	Z	98.4	0.083	0.90	9.3	0.70–1.10	0.074

Note: Notation as in Tables 1 and 4. References: Z, Zauke et al. (1995); R, Ritterhoff et al. (1996). Model predictions refer to gammarid collectives from the following rivers (see references above for details): J1, Q1, P1, J2, Q2, P2, and K from the Weser estuary, Ga from the Ems estuary, and Hu from the River Hunte. Regression constants statistically not different from zero; critical t values (two-sided), $t_{19;0.05} = 2.09$ (applies to observed G.s.-Gr, study 2); $t_{26;0.05} = 2.06$ (applies to observed G.l.-He, G.z.-Hu, and G.z.-Te, study 1), and $t_{29;0.05} = 2.04$ (applies to observed G.z.-Hu, study 2).

dency for regulation. Available data suggest that an incorporated Zn concentration of about 140 μg Zn·g dry weight^{-1} eventually represents an upper limit for the gammarids investigated. A tendency for Zn regulation was also described for *H. azteca* (Borgmann et al. 1993) and some decapode crustaceans (Rainbow 1993), whereas for *Gammarus pulex* and *Elminus modestus*, net accumulation was reported (Rainbow and White 1989; Rainbow 1993; Xu and Pascoe 1993). In the case of Hg, our finding cannot be interpreted as a tendency for regulation, but we are not able to present any sound explanation as yet.

In agreement with the present study, net accumulation of Cu was also reported for the amphipods *Echinogammarus pirloti* and *T. abyssorum* (Rainbow and White 1989; Ritterhoff and Zauke 1997*a*, 1997*b*). Borgmann and Norwood (1995) found a net uptake of Cu and Zn in *H. azteca* in short-term experiments, while in long-term experiments, the animals were able to regulate Cu. A net accumulation strategy was also reported for Pb in *T. abyssorum*, *Gammarus pseudolimnaeus*, and *H. azteca* (MacLean et al. 1996; Ritterhoff and Zauke 1997*a*, 1997*b*; Borgmann and Norwood 1999) and for Cd in various *Gammarus* species and *H. azteca* (Stuhlbacher and Maltby 1992; Borgmann et al. 1993; Zauke et al. 1995).

Implications for biomonitoring

As mentioned previously, the toxicokinetic studies presented in this paper are not intended as a tool to analyze mechanisms of metal uptake in organisms; rather, they are regarded as an integrated approach in biomonitoring, involving both field investigations and manipulative experiments (Zauke et al. 1996*b*). If possible, experiments under semicontrolled field conditions are recommended (Zauke et al. 1995), even if they are less reproducible. This goal prevents the application of radioactive isotopes and favours the application of metal mixtures instead of single-element dosing. Within this concept, it is necessary to specify the range of external metal exposures for which the models may serve as a predictive tool and to specify the sensitivity of the biomonitors to respond to a possible increase in metal bioavailability in the environment.

From Fig. 3, it may be deduced that Cu and Pb show a linear increase from the control level to 240 μg Cu·L^{-1} and 60 μg Pb·L^{-1}, while Zn tends to level off above 120 μg Zn·L^{-1} and Cd above 10 μg Cd·L^{-1}. Regarding mesozooplankton collectives from the Greenland Sea (*Calanus hyperboreus*, *T. abyssorum*, and *T. libellula*), two-compartment models were successfully verified up to exposure levels of 120–240 μg Cu·L^{-1}, 12–36 μg Pb·L^{-1}, and 400– 1200 μg Zn·L^{-1} (Ritterhoff and Zauke 1997*a*). If such a linear relationship is true, the toxicokinetic parameters should also be applicable to very low exposures.

However, the compartment models employed in this study, as in many other papers, assume that the metals accumulated in the field are not necessarily involved in the toxicokinetics due to the additive term C_0 (see eqs. 1 and 2). This is justified because in toxicokinetic studies, the organisms are normally exposed for a relatively short period to metal ions while in the field other metal species might be predominant and the metals taken up might be subject to subcellular sequestration (see discussions in Viarengo and Nott 1993; Dallinger 1995; Ritterhoff and Zauke 1998). Furthermore, other uptake routes such as food, particulate matter, or sediments have to be taken into account (e.g., Wang and Fisher 1998, 1999). When using compartment models as a predictive tool for waterborne metal uptake, the emphasis is on modelling a short-term response of organisms to a short-term increase in bioavailable soluble metal species. In this context, the sensitivity of prospective biomonitors to detect environmental changes is crucial: how large must an increase in exposure be to produce a detectable increase in metal concentrations in the organisms within routine biomonitoring programs?

The following calculations aim at providing a first approximation to this problem. The minimum difference that can be detected can be obtained from the equation

$$N = (t\ \text{value} \times \text{SD} / \Delta X)^2.$$

Assuming SD = 25%, $N = 3$, and the hypothetical experiment starting at C_0 values listed in Tables 4 and 5, the minimum difference that must be reached within 10-day uptake phase would be

$$\Delta X = 2.92/\sqrt{3} \times 0.25C_0 \approx 0.42C_0$$

Employing eq. 1 to predict metal uptake and using kinetic parameters listed in Table 4 and Table 8 (for Cd), it is possible to calculate C_W values necessary to exceed metal concentrations in gammarids at day 10 above $C_0 + \Delta X$ (viz. 90–140 μg Cu·g dry weight^{-1}, 70–100 μg Zn·g dry weight^{-1}, 3–5 μg Pb·g dry weight^{-1}, and 0.17–0.20 μg Cd·g dry weight^{-1}). The resulting C_W values can be regarded as the sensitivity of the biomonitors to detect an increase in metal bioavailability in the field, yielding exposures of approximately 30–40 μg Cu·L^{-1}, 70–150 μg Zn·L^{-1}, 2–3 μg Pb·L^{-1}, and 0.25–0.5 μg Cd·L^{-1}. These figures are clearly dependent on the C_0 values and on a good standard in the analytical procedures. Under other circumstances, they may be substantially higher.

Taking into account the exposure regime employed in toxicokinetic study 1 and the soluble metal concentrations to be expected in the field, the gammarids tested do not seem to be very sensitive regarding Cu and Zn. This is most probably due to the high metabolic requirements in crustaceans (Rainbow 1993). The sensitivity for Pb and Cd, on the other hand, seems to be rather high. Thus, toxicokinetic studies could eventually be performed under much lower exposures than in the present investigation.

Some authors argue that a successful verification of models involves comparison of model predictions not only with independent experimental data sets (see previous section) but also with results from field studies (e.g., Chapman 1995; Marinussen et al. 1997). Since we assume that accumulated metals under field conditions are eventually present in different chemical forms, it does not seem reasonable to compare experimental BCFs directly with BCFs calculated from field data (viz. metal concentration in organisms/metal concentrations in ambient water) without further evaluation. In our opinion, field verification rather requires a pronounced pollution gradient and eventually the application of the reciprocal transplant (active monitoring) approach (Walsh and Ohalloran 1998; Tedengren et al. 1999). Because such a pollution gradient is largely missing in our area, it was not possible to achieve a corresponding model verification.

In conclusion, our results show that the gammarid collectives tested may be used in biomonitoring studies and that the models obtained can serve as a predictive tool to assess environmental quality. This view is supported by a net accumulation strategy found for Cu, Cd, Pb, and, to lesser extent, Zn within the given constraints of our experimental design. Furthermore, two-compartment and logistic regression models could be successfully fitted for these metals in toxicokinetic study 1, leading to model constants statistically different from zero and thus to BCFs for the theoretical equilibrium. The toxicokinetic models obtained were successfully verified in toxicokinetic study 2 for Cu, Zn, Pb, and Cd. Only the G.l.-He model underestimates the observed data for Cd, most probably due to a salinity effect. Data for Cd were additionally verified with previously reported kinetic parameters, showing that not a unique set of k_1 and k_2 values exists that might be suitable as a prognostic tool, but rather a whole family of appropriate k_1 and k_2 combinations. Results for Hg indicate that the organisms tested may not be suitable as biomonitors for this element.

The calibration of gammarids as biomonitors was extended in toxicokinetic study 3 for Cu and Pb, showing a linear increase from the control level to 240 μg Cu·L^{-1} and 60 μg Pb·L^{-1}, while Zn tends to level off above 120 μg Zn·L^{-1} and Cd above 10 μg Cd·L^{-1}. These figures largely determine the range for which the models may serve as a predictive tool. A tentative estimation shows that the following increase in ambient waterborne metal concentrations might result in a detectable increase in accumulated metals in gammarids: 30–40 μg Cu·L^{-1}, 70–150 μg Zn·L^{-1}, 2–3 μg Pb·L^{-1}, and 0.25–0.5 μg Cd·L^{-1}. The gammarids tested do not seem to be very sensitive regarding Cu and Zn, in contrast with Pb and Cd.

Although the present paper provides information on accumulation of selected waterborne metals in gammarids within an integrated biomonitoring approach, current investigations in our department focus on metal uptake via food to assess the relative importance of both uptake routes by comparing the corresponding model parameters. Only when both pathways are fully evaluated will it be possible to develop compartment models as a more realistic predictive tool. Furthermore, future studies must address the incorporation of subcellular metal sequestration (e.g., granula, lysosomes, metallothioneins) in the modelling process, eventually leading to pharmacokinetic models.

Acknowledgements

This study was part of the Diploma Thesis of Birte Clason at the University of Oldenburg, partially funded by the "Hofrat Sack'sche Familienstiftung", Dortmund. We thank J. Ritterhoff, Oldenburg, for his help during field work and for helpful discussions. Valuable suggestions on a previous version of this manuscript were made by an anonymous referee and W.P. Norwood, Burlington.

References

Anonymous. 1994. Gemeinsames Bund/Länder-Meßprogramm für die Nordsee. Gewässergütemessungen im Küstenbereich der Bundesrepublik Deutschland, Gütedaten 1992/93. Niedersächsisches Umweltministerium, Hannover, Germany.

Anonymous. 1999. Chemikaliengesetz: Gesetz zum Schutz vor gefährlichen Stoffen. Anhang 1 (zu § 19a Abs. 1) — Grundsätze der guten Laborpraxis (GLP). *In* Handbuch angewandte Limnologie. Chap. IX-5, 7. Erg.Lfg. 4/99. *Edited by* C. Steinberg, H. Bernhardt, W. Calmano, H. Klapper, and R.-D. Wilken. ecomed, Landsberg am Lech.

Bates, D.M., and Watts, D.G. 1988. Nonlinear regression analysis and its applications. John Wiley & Sons, New York.

Bäumer, H.-P., van der Linde, A., and Zauke, G.-P. 1991. Structural equation models, applications in biological monitoring. Biometrie Inf. Med. Biol. **22**: 156–178.

Bernds, D., Wübben, D., and Zauke, G.-P. 1998. Bioaccumulation of trace metals in polychaetes from the German Wadden Sea: evaluation and verification of toxicokinetic models. Chemosphere, **37**: 2573–2587.

Bjerregaard, P., and Depledge, M.H. 1994. Cadmium accumulation in *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*: the influence of salinity and calcium ion concentrations. Mar. Biol. **119**: 385–395.

Borgmann, U., and Norwood, W.P. 1995. Kinetics of excess (above background) copper and zinc in *Hyalella azteca* and their relationship to chronic toxicity. Can. J. Fish. Aquat. Sci. **52**: 864–874.

Borgmann, U., and Norwood, W.P. 1999. Assessing the toxicity of lead in sediments to *Hyalella azteca*: the significance of bioaccumulation and dissolved metal. Can. J. Fish. Aquat. Sci. **56**: 1494–1503.

Borgmann, U., Ralph, K.M., and Norwood, W.P. 1989. Toxicity test procedures for *Hyalella azteca*, and chronic toxicity of cadmium and pentachlorophenol to *H. azteca*, *Gammarus fasciatus*, and *Daphnia magna*. Arch. Environ. Contam. Toxicol. **18**: 756–764.

Borgmann, U., Norwood, W.P., and Clarke, C. 1993. Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*. Hydrobiologia, **259**: 79–89.

Büttner, J.R., Borth, R., Boutwell, H.J., Broughton, P.M.G., and Bowyer, R.C. 1980. Approved recommendation (1978) on quality control in clinical chemistry. J. Clin. Chem. Clin. Biochem. **18**: 78–88.

Chapman, P.M. 1995. Extrapolating laboratory toxicity results to the field. Environ. Toxicol. Chem. **14**: 927–930.

Dallinger, R. 1995. Mechanisms of metal incorporation into cells. *In* Cell biology in environmental toxicology. *Edited by* M.P. Cajaraville. Universidad del Pais Vasco, Bilbao. pp. 135–154.

DIN-32645. 1994. Nachweis-, Erfassungs- und Bestimmungsgrenze. *In* Chemische Analytik. *Edited by* Arbeitsausschuß chemische Terminologie. Beuth Verlag, Berlin.

Haarich, M., and Schmidt, D. 1993. Schwermetalle in der Nordsee (2): Ergebnisse der ZISCH-Grossaufnahme vom 28.1. bis 6.3.1987. Dtsch. Hydrogr. Z. **45**: 371–431.

Holzbecher, E. 1997. Testing ecological models: the meaning of validation — remarks. Ecol. Model. **102**: 375–377.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54**: 187–211.

Hurlbert, S.H., and White, M.D. 1993. Experiments with freshwater invertebrate zooplanktivores: quality of statistical analyses. Bull. Mar. Sci. **53**: 128–153.

Lincoln, R.J. 1979. British marine Amphipoda: Gammaridea. British Museum (Natural History), London, U.K.

MacLean, R.S., Borgmann, U., and Dixon, D.G. 1996. Bioaccumulation kinetics and toxicity of lead in *Hyalella azteca* (Crustacea, Amphipoda). Can. J. Fish. Aquat. Sci. **53**: 2212–2220.

Marinussen, M.P.J.C., Vanderzee, S.E.A.T.M., and Dehaan, F.A.M. 1997. Cu accumulation in *Lumbricus rubellus* under laboratory conditions compared with accumulation under field conditions. Ecotoxicol. Environ. Saf. **36**: 17–26.

Meurs, H.-G., and Zauke, G.-P. 1996. Populationsbiologische Untersuchungen an Gammariden als Grundlage zur Abschätzung von Eingriffen (UVS) in Flußmündungen. Z. Ökol. Naturschutz. **5**: 107–113.

Naylor, C., Maltby, L., and Calow, P. 1989. Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. Hydrobiologia, **188/189**: 517–523.

OECD. 1981. Guideline for testing chemicals. OECD, Paris, France.

Rainbow, P.S. 1993. The significance of trace metal concentration in marine invertebrates. *In* Ecotoxicology of metals in invertebrates. *Edited by* R. Dallinger and P.S. Rainbow. Lewis Publishers, Boca Raton, Fla. pp. 4–23.

Rainbow, P.S. 1995*a*. Biomonitoring of heavy metal availability in the marine environment. Mar. Pollut. Bull. **31**: 183–192.

Rainbow, P.S. 1995*b*. Physiology, physicochemistry and metal uptake — a crustacean perspective. Mar. Pollut. Bull. **31**: 55–59.

Rainbow, P.S., and White, S.L. 1989. Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an amphipod and a barnacle. Hydrobiologia, **174**: 245–262.

Rainbow, P.S., Phillips, D.J.H., and Depledge, M.H. 1990. The significance of trace metal concentrations in marine invertebrates, a need for laboratory investigation of accumulation strategies. Mar. Pollut. Bull. **21**: 321–324.

Rinderhagen, M., Ritterhoff, J., and Zauke, G.-P. 2000. Crustaceans as bioindicators. *In* Biomonitoring of polluted water — reviews on actual topics. *Edited by* A. Gerhardt. Environmental Research Forum. Vol. 9. Trans Tech Publications – Scitech Publications, Uetikon-Zuerich. pp. 161–194.

Ritterhoff, J., and Zauke, G.-P. 1997*a*. Bioaccumulation of trace metals in Greenland Sea copepod and amphipod collectives on board ship: verification of toxicokinetic model parameters. Aquat. Toxicol. **40**: 63–78.

Ritterhoff, J., and Zauke, G.-P. 1997*b*. Evaluation of trace metal toxicokinetics in Greenland Sea copepod and amphipod collectives from semi-static experiments on board ship. Polar Biol. **17**: 242–250.

Ritterhoff, J., and Zauke, G.-P. 1997*c*. Trace metals in field samples of zooplankton from the Fram Strait and the Greenland Sea. Sci. Total Environ. **199**: 255–270.

Ritterhoff, J., and Zauke, G.-P. 1998. Potential role of metal- binding proteins in cadmium detoxification in *Themisto libellula* (Mandt) and *Themisto abyssorum* Boeck from the Greenland Sea. Mar. Environ. Res. **45**: 179–191.

Ritterhoff, J., Zauke, G.-P., and Dallinger, R. 1996. Calibration of the estuarine amphipods, *Gammarus zaddachi* Sexton (1912), as biomonitors: toxicokinetics of cadmium and possible role of inducible metal binding proteins in Cd detoxification. Aquat. Toxicol. **34**: 351–369.

Rykiel, E.J. 1996. Testing ecological models: the meaning of validation. Ecol. Model. **90**: 229–244.

Stuhlbacher, A., and Maltby, L. 1992. Cadmium resistence in *Gammarus pulex* (L.). Arch. Environ. Contam. Toxicol. **22**: 319–324.

Tappin, A.D., Millward, G.E., Statham, P.J., Burton, J.D., and Morris, A.W. 1995. Trace metals in the central and southern North Sea. Estuarine Coastal Shelf Sci. **41**: 275–323.

Tedengren, M., Olsson, B., Bradley, B., and Zhou, L.Z. 1999.

Heavy metal uptake, physiological response and survival of the blue mussel (*Mytilus edulis*) from marine and brackish waters in relation to the induction of heat-shock protein 70. Hydrobiologia, **393**: 261–269.

Viarengo, A., and Nott, J.A. 1993. Mini-review. Mechanisms of heavy metal cation homeostasis in marine invertebrates. Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol. **104**: 355–372.

Walsh, A.R., and Ohalloran, J. 1998. Accumulation of chromium by a population of mussels (*Mytilus edulis* (L.)) exposed to leather tannery effluent. Environ. Toxicol. Chem. **17**: 1429–1438.

Wang, W.X., and Fisher, N.S. 1998. Accumulation of trace elements in a marine copepod. Limnol. Oceanogr. **43**: 273–283.

Wang, W.X., and Fisher, N.S. 1999. Delineating metal accumulation pathways for marine invertebrates. Sci. Total Environ. **238**: 459–472.

Wang, X.L., Ma, Y.J., Yu, W.J., and Geyer, H.J. 1997. Two- compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by algae: quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. Chemosphere, **35**: 1781–1797.

Wilkinson, L. (*Editor*). 1998. Systat 8.0 statistics. SPSS Inc., Chicago, Ill.

Wright, D.A. 1995. Trace metal and major ion interactions in aquatic animals. Mar. Pollut. Bull. **31**: 8–18.

Xu, Q., and Pascoe, D. 1993. The bioconcentration of zinc by *Gammarus pulex* (L.) and the application of a kinetic model to determine bioconcentration factors. Water Res. **27**: 1683–1688.

Zauke, G.-P., von Lemm, R., Meurs, H.-G., and Butte, W. 1995. Validation of estuarine gammarid collectives (Amphipoda: Crustacea) as biomonitors for cadmium in semi-controlled toxicokinetic flow-through experiments. Environ. Pollut. **90**: 209–219.

Zauke, G.-P., Krause, M., and Weber, A. 1996*a*. Trace metals in mesozooplankton of the North Sea: concentrations in different taxa and preliminary results on bioaccumulation in copepod collectives (*Calanus finmarchicus* / *C. helgolandicus*). Int. Rev. Gesamten Hydrobiol. **81**: 141–160.

Zauke, G.-P., Petri, G., Ritterhoff, J., and Meurs, H.-G. 1996*b*. Theoretical background for the assessment of the quality status of ecosystems: lessons from studies of heavy metals in aquatic invertebrates. Senckenb. Marit. **27**: 207–214.

Zauke, G.-P., Meurs, H.-G., Todeskino, D., Kunze, S., Bäumer, H.-P., and Butte, W. 1988. Zum Monitoring von Cadmium, Blei, Nickel, Kupfer und Zink in Balaniden (Cirripedia: Crustacea), Gammariden (Amphipoda: Crustacea) und Enteromorpha (Ulvales: Chlorophyta). UBA-Texte 18/88. Umweltbundesamt, Berlin.

Effect of unstable aluminium chemistry on Arctic char (*Salvelinus alpinus*)

Antonio B.S. Poléo and Frode Bjerkely

Abstract: Arctic char (*Salvelinus alpinus*) were exposed to a non-steady-state Al-rich medium (pH 5.8, total Al 480 $\mu g \cdot L^{-1}$, total organic C 3.0 mg $C \cdot L^{-1}$, Ca^{2+} 2.9 $mg \cdot L^{-1}$, temperature 7.5°C). An Al-poor medium (pH 5.2 and 5.8, total Al 99 $\mu g \cdot L^{-1}$, total organic C 3.0 mg $C \cdot L^{-1}$, Ca^{2+} 2.9 $mg \cdot L^{-1}$, temperature 8.0°C) acted as control. The Al-rich medium was acutely toxic to the Arctic char. Total mortality (percent) and mortality rate (LT_{50}) were highest in fish exposed to the Al-rich medium immediately after mixing and decreased systematically with water residence time (e.g., from 65 h at 1 min to 124 h at 3 min to more than 400 h at 15 min). Gill morphology changes, Al gill deposition, and haematological parameters revealed significant effects of Al, which all correlated with water residence time. Signs of acclimation or high difference in Al tolerance between individuals were observed. This indicates that effects of Al in fish are dependent on the degree of Al polymerisation and supports earlier indications that Arctic char are relatively tolerant of acidic Al-rich water.

Résumé : Des spécimens d'omble chevalier (*Salvelinus alpinus*) ont été exposés à un milieu non stationnaire riche en aluminium (pH 5,8, Al total 480 $\mu g \cdot L^{-1}$, carbone organique total (COT) 3,0 mg $C \cdot L^{-1}$, Ca^{2+} 2,9 $mg \cdot L^{-1}$, temperature 7,5°C). Un milieu pauvre en Al (pH 5,2 et 5,8, Al total 99 $\mu g \cdot L^{-1}$, COT 3,0 mg $C \cdot L^{-1}$, Ca^{2+} 2,9 $mg \cdot L^{-1}$, temperature 8,0°C) servait de témoin. Le milieu riche en Al s'est révélé d'une toxicité aiguë pour l'omble chevalier. La mortalité totale (%) et le taux de mortalité (TL_{50}) étaient les plus élevés chez les poissons exposés au milieu riche en Al juste après le mélange, puis baissaient systématiquement avec la durée du séjour dans l'eau (p. ex. de 65 h à 1 min, 124 h à 3 min, à plus de 400 h à 15 min). Des changements dans la morphologie des branchies, le dépôt d'Al sur les branchies et les paramètres hématologiques révélaient des effets significatifs de l'aluminium, qui étaient tous corrélés avec la durée du séjour dans l'eau. On a observé des signes d'acclimatement ou une nette différence dans la tolérance à Al d'un individu à l'autre. Cela indique que les effets de l'aluminium chez les poissons dépendent du degré de polymérisation favorisé par ce métal, et confirme que l'omble chevalier tolère relativement bien les eaux acides riches en Al.

[Traduit par la Rédaction]

Introduction

The most significant consequence of the acidification of soil water systems is the mobilisation of Al from the edaphic to the aquatic environment (Cronan and Schofield 1979; Driscoll and Postek 1996; Gensemer and Playle 1999). Aqueous Al is recognised by many authors as the principal toxicant killing fish in acidified surface waters (reviewed by Sparling and Lowe 1996; Gensemer and Playle 1999). As a result, many attempts have been made to relate the Al toxicity to the levels of various aqueous Al forms that can occur in the aquatic environment. Present understanding of the effect of Al in natural waters and its interactions with aquatic biota is based largely on chemical equilibrium constants. The environment, however, in particular the biological environment, is not in steady state. Accordingly, true chemical equilibrium is seldom approached in natural systems like surface waters, and some ecotoxicological studies have indicated that a non-steady-state Al chemistry can predominate in these systems (e.g., Rosseland et al. 1992; Lydersen et al. 1994; Poléo et al. 1994). The same studies also indicate that a non-steady-state transient Al chemistry may dictate the Al toxicity to fish in acidified freshwater systems.

Based on this and other observations, Poléo (1995) suggested that the process of Al polymerisation is of major importance for the mechanism of acute toxicity of Al in fish, especially after a rise in pH or temperature. This proposed mechanism might involve the binding of monomeric and transient cationic Al species to gill mucus and membranes, with a subsequent Al polymerisation and accumulation on the gill surface (Lydersen et al. 1994; Poléo 1995). Furthermore, it has been hypothesised that the primary effect of the Al polymerisation taking place on the gill surface is acute hypoxia due to clogging of the gill interlamellar spaces (Poléo et al. 1994; Poléo 1995; Witters et al. 1996). The conditions for Al polymerisation are optimal when pH is between 5.0 and 7.0, depending on water temperature (Hem and Roberson 1967; Lydersen et al. 1991). According to this, it has been documented previously that hypoxia seems to be the main cause of acute toxicity in fish exposed to aqueous Al at relatively high pH, between 5.0 and 6.5 (Neville 1985).

Because little is known about the morphological and physiological effects of a transient Al chemistry affecting the fish gills, we have investigated this in more detail in the

Received June 25, 1999. Accepted April 14, 2000.
J15214

A.B.S. Poléo.[1] Department of Biology, University of Oslo, P.O. Box 1066, Blindern, N-0316 Oslo, Norway.
F. Bjerkely. Kvaerner Oil & Gas, P.O. Box 1071, N-3204 Sandefjord, Norway.

[1] Author to whom all correspondence should be addressed. e-mail: toni.poleo@bio.uio.no

Fig. 1. Schematic presentation of the experimental setup. PP, peristaltic pump.

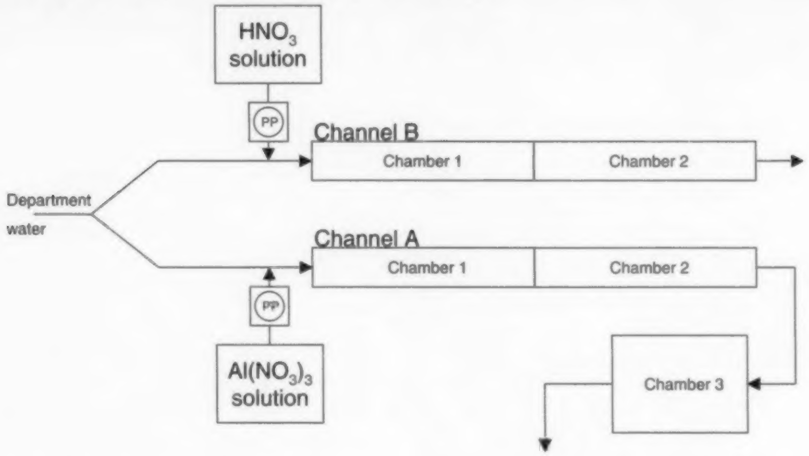


Table 1. Water quality parameters of the water in the fish-holding department at the University of Oslo.

	Mean ± SD	*n*
pH	6.4±0.1	35
Conductivity (μS·cm^{-1})	23±4	35
Na$^+$ (mg·L^{-1})	1.97±0.16	5
K$^+$ (mg·L^{-1})	0.35±0.02	5
Ca^{2+} (mg·L^{-1})	2.85±0.25	5
Mg^{2+} (mg·L^{-1})	0.49±0.04	5
SO$_4^{2-}$ (mg·L^{-1})	5.64±0.47	5
Cl$^-$ (mg·L^{-1})	2.38±0.27	5
F$^-$ (μg·L^{-1})	80±2	5
Total N (mg N·L^{-1})	0.36±0.09	5
SiO$_2$ (mg·L^{-1})	1.54±0.09	5
Fe (μg·L^{-1})	10±4	12
Total organic C (mg C·L^{-1})	3.00±0.07	5

present study. We tested the prediction that fish mortality, the extent of gill damage, and the degrees of physiological disturbances are correlated with the degree of ongoing Al polymerisation. A major problem when describing the mechanism of acute Al toxicity to fish, however, has been to decide which biological symptoms are primary effects of Al and which symptoms are secondary effects. Thus, in the present study, we also tested the hypothesis that hypoxia is the primary physiological effect of a transient Al chemistry, due to Al polymerisation and accumulation on the gill surface. The Arctic char (*Salvelinus alpinus*) is a relatively common freshwater fish species in acidified areas of Scandinavia. Despite this, the toxicity of aqueous Al has so far not been studied in any detail in this salmonid.

Materials and methods

Experimental animals

One-year-old Arctic char (1+) of about 30 g were obtained from the Research Station for Freshwater Fish at Ims, Rogaland, southwest Norway. The fish were brought into the fish-holding department at the University of Oslo where they were kept and acclimatised for at least 2 months in Oslo city tap water (Table 1) before the experiments. The fish were not fed 3 days before and during experiments to avoid the influence of food on blood parameters.

Test conditions

Two test media with different Al concentrations were prepared by addition of HNO$_3$ and Al(NO$_3$)$_3$·9H$_2$O to the fish-holding department water. An Al-rich medium (nominal pH 5.8) was prepared by the addition of an Al(NO$_3$)$_3$ solution. The pH of this Al(NO$_3$)$_3$ solution was kept low (pH 2.0) to ensure that the major amount of Al was present as Al^{3+} before it was added to the department water. An Al-poor medium (control, nominal pH 5.2 and 5.8) was also prepared by the addition of HNO$_3$ only. Untreated water (pH 6.4) acted as the overall reference medium. Water temperature was kept between 6.0 and 8.0°C during the experiments.

We used a flow-through exposure system for the experiments (Fig. 1). The department water was led into two exposure channels, A and B (180 cm long, 20 cm wide, and 15 cm deep), which were divided into two chambers, 1 and 2, of equal size (90 × 20 × 15 cm). At the end of channel A, the water was led to a third exposure chamber (chamber 3, 40 × 40 × 15 cm). The chemicals were added to the water at the inlet of the channels (Fig. 1). Thus, in channel A, fish were exposed to a transient Al chemistry due to a rapid rise in pH, from 2.0 to about 5.8, after mixing the Al solution with department water. The water flow rate into the exposure channels was approximately 4 L·min^{-1}. The age of the media in the first exposure chambers was about 1 min and in chamber 2 was about 3 min. The total residence time through channel A and the additional chamber 3 was 15 min.

Experimental protocol

Mortality rates of Arctic char exposed to the Al-rich medium (nominal pH 5.8) were evaluated. In channel A, we used 60 fish equally distributed to the three exposure chambers. In channel B, the control exposure (Al-poor medium, nominal pH 5.2), we used only 20 fish placed in the second chamber. Mortality was quantified in terms of LT$_{50}$. Fish were judged to be dead when opercular movements ceased and no swimming response could be elicited by stimulation of the caudal peduncle. The exposures were carried out within a period of 2 weeks.

In a separate experiment, the effects of a transient Al chemistry on various morphological and physiological parameters were evaluated. The fish were placed into the chambers 3 days before the exposures. In the exposure to the Al-rich medium (channel A, nominal pH 5.8), we used 25 fish in chamber 1 and 20 fish in chambers 2 and 3. In the Al-poor medium (channel B, nominal pH 5.8), we used 20 fish placed in the second chamber. Immediately before the exposures were started, the first blood and gill samples were taken from five fish from chamber 1 in channel A (time zero), i.e., the reference group. After this, based on the observations from the mortality trials, blood and gill samples were taken after 1, 2, 3, and 5 days from five fish from each exposure chamber.

Water temperature, conductivity, and pH were measured daily throughout the experimental period. The chemical dose and water flow into the channels were also controlled each day. During the experiments, aqueous Al was analysed between two and eight times, depending on the duration of the exposures. The pH and conductivity measurements were performed immediately after the water samples were taken. The Al analyses were performed in situ at the channels, according to Lydersen et al. (1994). Before we started the experiments, we also performed a characterisation of the Al chemistry of channel A.

Analytical techniques

The analyses of water pH, conductivity, and general chemical composition were performed exactly as described by Poléo et al. (1997). Aqueous Al was fractionated by the HQ-MIBK extraction technique described by Barnes (1975) combined with the cation-exchange procedure described by Driscoll (1984) according to the protocol described by Poléo et al. (1997).

Fish were stunned with a blow to the head before sampling. Blood samples were taken by cardiac puncture into 1-mL heparinised syringes and centrifuged for 5 min. Haematocrit (percent red blood cells) was measured and the plasma samples were immediately analysed for Cl^-. Plasma Cl^--concentration was determined coulometrically using a Radiometer CMT-10 chloride titrator with an expected precision of ±0.5%. Plasma samples were stored at –80°C for later Na^+ and lactate analyses. Plasma Na^+ concentration was determined by atomic absorption spectrophotometry. The atomic absorption spectrophotometry detection limit was 0.15 ppm. Plasma lactate concentration was measured enzymatically with a standard analysing kit (Sigma kit 735-10). The standard deviation of the lactate measurements was calculated to be ±7.2%.

Gill samples were taken immediately after the blood samples to avoid post mortem artefacts. Whole gill arches were fixed in a mixture of 1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for scanning electron microscopy. The gills were then postfixed in 1% OsO_4, dehydrated in a graded series of ethanol, and critical point dried using carbon dioxide. Each gill arch was finally mounted on stubs using epoxy glue, sputter-coated with gold–palladium, and examined in a JEOL 6400 scanning electron microscope.

Gills were scored on a scale from 0 to 3 for relative levels of gill damage according to Schofield and Trojnar (1980). A score of 0 indicated no visible damage. Cell proliferation and clubbing at the distal ends of the filaments was given a score of 1. Extensive clubbing and lamellar fusion was assigned a score of 2. Finally, epithelial desquamation and general loss of gill structure was given a score of 3. In addition, one gill arch from each fish was collected, weighed, and dissolved in 1 mL of concentrated HNO_3 at 50°C for determination of the total Al content by graphite furnace atomic absorption spectrophotometry.

Results

Water chemistry

The Al-rich medium pH was 5.8 ± 0.1 (mean ± SD, n = 21) throughout the experiments (Table 2). The pH in the Al-poor medium was 5.3 ± 0.2 (n = 21) in the mortality experiment and 6.1 ± 0.1 (n = 4) in the physiological experiment. The pH of the reference water was 6.4 ± 0.1 (n = 35). The mean water temperature was approximately 7.5°C in the Al-rich medium, between 7.5 and 8.4°C in the Al-poor medium, and 6.4°C in the reference water. The electrical conductivity in the various media was relatively stable, between 23 and 29 $\mu S\cdot cm^{-1}$ throughout the experiments (Table 2).

During experiments, the Al-rich medium had a total Al (Al_r) concentration of about 480 $\mu g\cdot L^{-1}$, which was stable through the channel and chamber 3 (Table 2). The Al fractions in this medium, however, changed through the channel. In chamber 1, approximately 390 $\mu g\ Al_r\cdot L^{-1}$ was present as monomeric Al species (Al_a). This fraction declined to about 330 $\mu g\cdot L^{-1}$ in chamber 3 ($p < 0.0005$). These changes resulted in a corresponding, and significant, increase in the calculated amount of Al colloids (Al_c) ($p < 0.0005$). The organic monomeric Al fraction (Al_o) also increased significantly ($p < 0.0005$). This gave a significant decrease in the calculated amount of inorganic monomeric Al (Al_i) from 214 ± 19 $\mu g\cdot L^{-1}$ (n = 8) in chamber 1 to 134 ± 21 $\mu g\cdot L^{-1}$ (n = 8) in chamber 3 ($p < 0.01$) (Table 2). The Al_r concentration in the untreated water was 99 ± 9 $\mu g\cdot L^{-1}$ (n = 12). Most of this was in unavailable colloidal or organic Al forms, while only 8 ± 5 $\mu g\cdot L^{-1}$ (n = 12) was present as Al_i (Table 2). The chemical characterisation of channel A prior to the experiments confirmed the observations made during fish experiments. The Al-rich water had an Al_r concentration of about 480 $\mu g\cdot L^{-1}$, which did not change through the channel, while the various Al fractions did change gradually on their way through it (see Fig. 2).

Fish mortality

The Al-rich medium was acutely toxic to Arctic char, and both mortality (percent) (Fig. 3) and mortality rate (LT_{50}) correlated with water residence time. The mortality rate was highest in the first chamber (LT_{50} = 65 h) and decreased systematically from chamber 1 to chamber 3. LT_{50} in chamber 2 was 124 h, while in chamber 3, only 20% of the fish died. In chamber 2, the initial mortality rate was relatively high, but after approximately 140 h of exposure, when 60% of the fish had died, the mortality more or less came to an end (Fig. 3). Only one fish died in this chamber during the next 260 h of exposure. In chamber 3, the same tendency was evident, and the mortality was low. No mortality of fish was observed in the control.

In the physiological experiment, fish mortality was also highest in chamber 1. In this chamber, no fish survived to the last sampling, i.e., for 120 h. In chambers 2 and 3, a few individuals were dead after 72 h of exposure, but no further mortality was observed during the last 48 h of the experiment.

Aluminium deposition on the gills

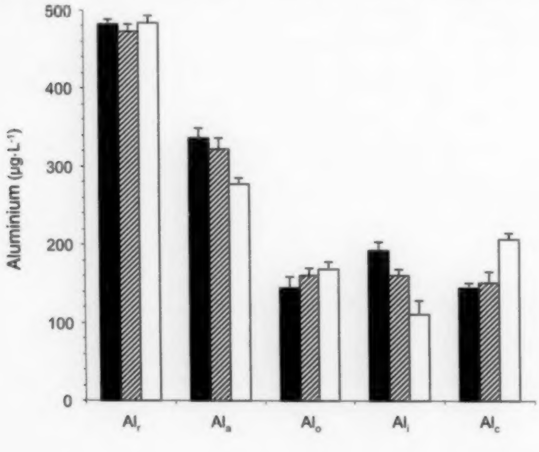
Substantial amounts of Al were deposited on the gills in fish exposed to the Al-rich medium (pH 5.8) (Fig. 4). This amount correlated with the water residence time. After 2 days in the first chamber, Al deposition on the gills was 470 ± 88 $\mu g\ Al\cdot g$ wet weight^{-1} (mean ± SE, n = 5) compared with 264 ± 44 (n = 5) and 202 ± 20 $\mu g\ Al\cdot g$ wet weight^{-1} (n = 5) in chambers 2 and 3, respectively. The amount of Al on the gills was also dependent on exposure time and increased rapidly in all three chambers during the first 2 days (Fig. 4). After this, we observed a small but not statistically significant decrease in deposited Al towards the end of the

Table 2. Aluminium chemistry data for the different media from the experimental period.

	Al rich, nominal pH 5.8	Al poor, nominal pH 5.2	Al poor, nominal pH 5.8	Hatchery water
pH				
In	5.8±0.1 (21)	5.3±0.2 (7)	6.1±0.1 (4)	6.4±0.1 (35)
Out	5.8±0.1 (21)	5.3±0.3 (7)		
Temperature (°C)				
In	7.4±1.8 (21)	8.3±0.8 (7)	7.5±0.2 (4)	6.4±1.2 (35)
Out	7.5±0.5 (21)	8.4±0.5 (7)		
Conductivity (μS·cm^{-1})				
In	25±4 (21)	28±5 (7)	29±1 (4)	23±4 (35)
Out	25±4 (21)	28±2 (7)		
$\mathrm{Al_r}$ (μg·L^{-1})				
In	482±26 (8)			99±9 (12)
Out	477±17 (8)			
$\mathrm{Al_a}$ (μg·L^{-1})				
In	394±19 (8)***			52±5 (12)
Out	329±15 (8)			
$\mathrm{Al_o}$ (μg·L^{-1})				
In	180±6 (8)*			45±6 (12)
Out	195±14 (8)			
$\mathrm{Al_i}$ (μg·L^{-1})				
In	214±19 (8)***			8±5 (12)
Out	134±21 (8)			
$\mathrm{Al_c}$ (μg·L^{-1})				
In	88±20 (8)***			47±7 (12)
Out	148±20 (8)			

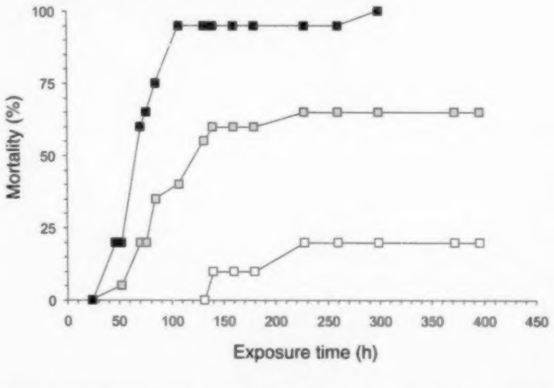
Note: "In" values represent a residence time of 1 min and "out" values represent a water residence time of 15 min. Means ± SD are shown (number of observations in parentheses). Statistically significant changes through the channels are indicated (*t* test: $*p < 0.01$; $***p < 0.0005$). $\mathrm{Al_r}$, total Al; $\mathrm{Al_a}$, total monomeric Al; $\mathrm{Al_o}$, organic monomeric Al; $\mathrm{Al_i}$, inorganic monomeric Al; $\mathrm{Al_c}$, colloidal Al.

Fig. 2. Amounts of various Al fractions (means ± SD, $n = 3$) determined in channel A before the experiments were started. Water residence time: solid bars, 1 min; hatched bars, 3 min; open bars, 15 min. $\mathrm{Al_r}$, total Al; $\mathrm{Al_a}$, total monomeric Al; $\mathrm{Al_o}$, organic monomeric Al; $\mathrm{Al_i}$, inorganic monomeric Al; $\mathrm{Al_c}$, colloidal Al.



exposure in all three chambers. No Al deposition was detected on gills from fish exposed to the Al-poor medium (pH 6.1) or in the reference group (pH 6.4), i.e., the background values were between 1 and 2 μg Al·g wet weight^{-1} ($n = 5$ for both groups).

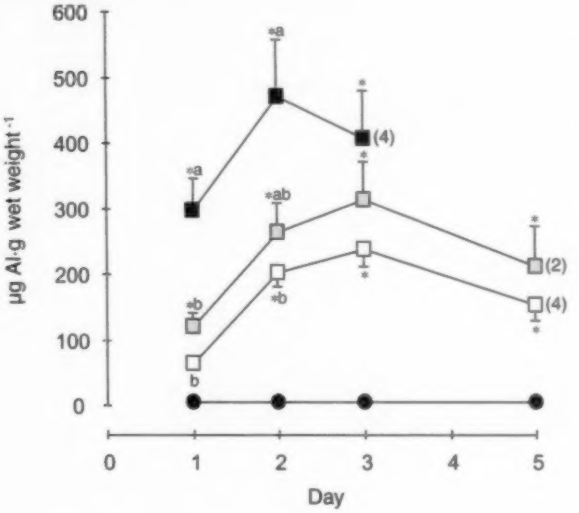
Fig. 3. Cumulative mortality of Arctic char exposed to various degrees of Al ageing. Water residence time: solid squares, 1 min (chamber 1); hatched squares, 3 min (chamber 2); open squares, 15 min (chamber 3). $n = 20$ for each curve.



Blood and plasma parameters

Blood haematocrit increased in fish exposed to the Al-rich medium, and the increase was successively more rapid and reached higher values in fish from chamber 1 to chamber 3 (Fig. 5). Haematocrit was 37 ± 2% ($n = 5$) in the reference

Fig. 4. Aluminium deposition on gills from Arctic char exposed to the Al-rich medium (squares) and the Al-poor medium (control (circles)) (means ± SE, $n = 5$ if not stated otherwise on the graph). Water residence time: solid squares, 1 min; hatched squares, 3 min; open squares, 15 min. An asterisk indicates a significant difference from the control (one-way ANOVA, Tukey–Kramer comparison, $p < 0.05$). Significant differences ($p < 0.05$) among treatments at one sampling period are indicated by treatment groups that do not share a common letter. If no letters are present for the sampling period, there were no significant differences between means.



group and increased to 54 ± 2% ($n = 4$) after 1 day in chamber 1, to 51 ± 4% ($n = 3$) after 2 days in chamber 2, and to 50 ± 3% ($n = 5$) after 3 days in chamber 3 (Fig. 5A). Blood haematocrit was stable and varied between 36 ± 3 ($n = 5$) and 38 ± 2% ($n = 5$) in fish exposed to the Al-poor medium.

Plasma ions decreased in fish exposed to the Al-rich medium, with a systematic increase in the rate of loss with decreasing water residence time (Figs. 5C and 5D). In chamber 1, plasma Cl^- decreased to 86 ± 3 $mmol \cdot L^{-1}$ ($n = 3$) after 1 day and to 69 ± 9 $mmol \cdot L^{-1}$ ($n = 3$) after 3 days. In chambers 2 and 3, plasma Cl^- decreased gradually to 77 ± 12 ($n = 4$) and 96 ± 12 $mmol \cdot L^{-1}$ ($n = 5$), respectively, after 3 days (Fig. 5C). Plasma Cl^- was stable in fish exposed to the Al-poor medium, varying between 120 ± 6 ($n = 5$) and 124 ± 1 $mmol \cdot L^{-1}$ ($n = 4$). In the reference group, plasma Cl^- was 123 ± 2 $mmol \cdot L^{-1}$ ($n = 5$).

In chamber 1, plasma Na^+ decreased to 94 ± 7 $mmol \cdot L^{-1}$ ($n = 3$) after 1 day and to 86 ± 3 $mmol \cdot L^{-1}$ ($n = 5$) after 2 days. In chambers 2 and 3, plasma Na^+ decreased gradually to 95 ± 8 ($n = 4$) and 104 ± 4 $mmol \cdot L^{-1}$ ($n = 5$), respectively, after 3 days of exposure (Fig. 5D). After the first day of exposure to the Al-poor medium, plasma Na^+ was lower (130 ± 3 $mmol \cdot L^{-1}$, $n = 5$) than in the reference group (143 ± 2 $mmol \cdot L^{-1}$, $n = 5$). However, plasma Na^+ remained relatively stable in the Al-poor medium during the rest of the experiment, between 128 ± 3 ($n = 4$) and 131 ± 6 $mmol \cdot L^{-1}$ ($n = 5$).

Table 3. Levels of macroscopic gill lesions in Arctic char from the various exposures examined by scanning electron microscopy.

	Exposure time (days)			
Medium	0	1	2	3
Reference group	0 ($n = 5$)			
Al poor			0 ($n = 4$)	
Al rich, 1 min		2 ($n = 4$)	3 ($n = 5$)	3 ($n = 3$)
Al rich, 3 min		1 ($n = 5$)	2 ($n = 3$)	3 ($n = 4$)
Al rich, 15 min		0 ($n = 5$)	1 ($n = 5$)	2 ($n = 5$)

Note: See Materials and methods for an explanation of the scores; higher numbers indicate greater damage. Fish exposed for 5 days were not examined.

Plasma lactate increased in fish exposed to the Al-rich medium in chambers 1 and 2 but was only significantly higher than in control fish in chamber 1 after 2 days of exposure (Fig. 5B). In chamber 1, plasma lactate increased to 3.3 ± 0.9 $mmol \cdot L^{-1}$ ($n = 4$) after 1 day and further to 4.7 ± 1.0 $mmol \cdot L^{-1}$ ($n = 5$) after 2 days. In chamber 2, plasma lactate showed a small, but not significant, increase to 2.6 ± 0.6 $mmol \cdot L^{-1}$ ($n = 4$) after 3 days of exposure. In chamber 3, no increase in plasma lactate was evident, and it varied between 1.1 ± 0.0 ($n = 5$) and 1.8 ± 0.5 $mmol \cdot L^{-1}$ ($n = 5$). In the Al-poor medium, plasma lactate varied between 1.1 ± 0.1 ($n = 5$) and 1.6 ± 0.3 $mmol \cdot L^{-1}$ ($n = 5$). Plasma lactate was 1.0 ± 0.1 $mmol \cdot L^{-1}$ ($n = 5$) in the reference group.

In all groups, blood and plasma parameters seemed to recover during the last period of the exposures (Fig. 5). However, except from haematocrit in fish from chamber 3, this recovery in blood and plasma parameters was not statistically significant (one-way analysis of variance (ANOVA), $p > 0.05$).

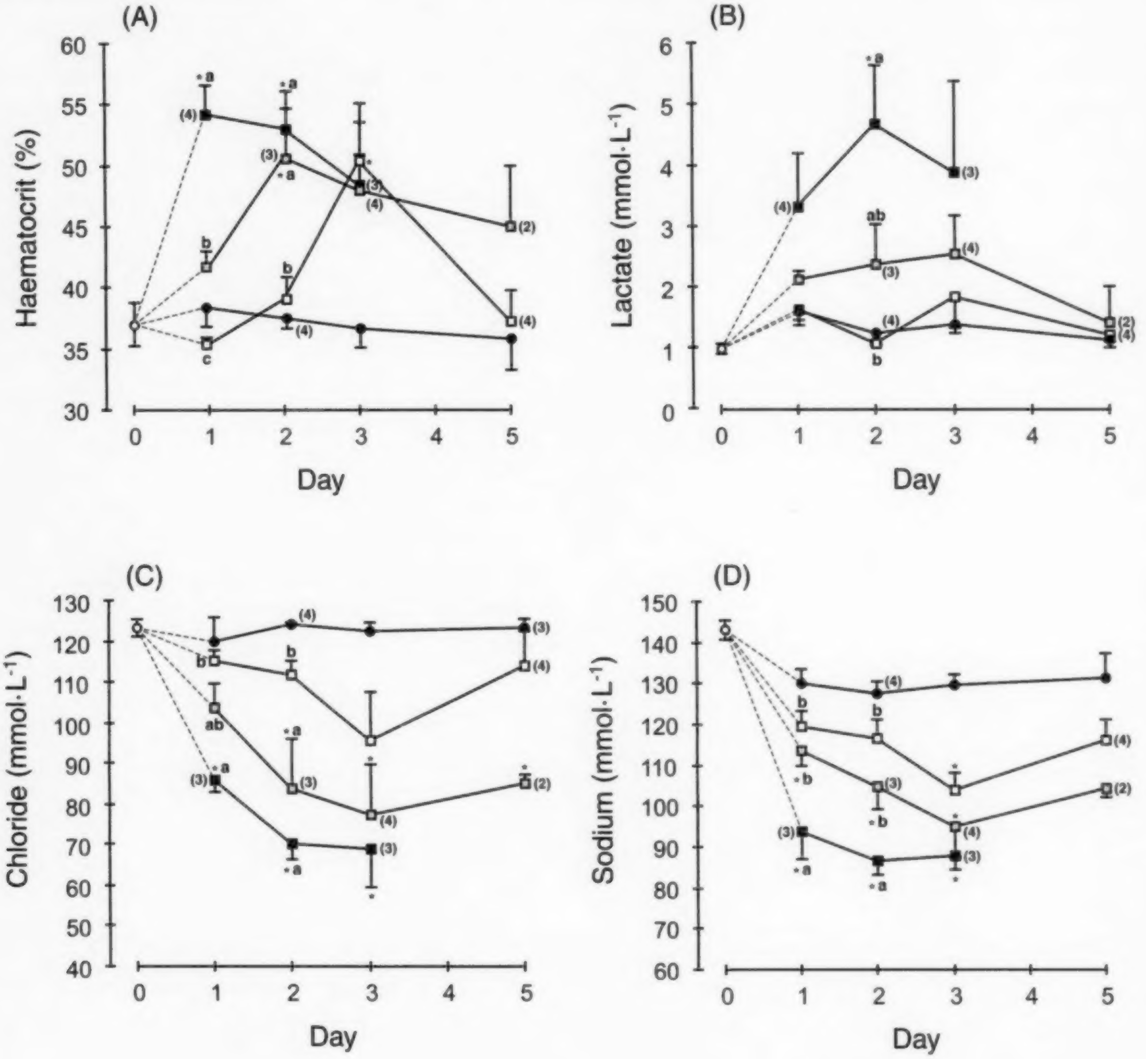
Gill morphology

Structural changes in the gills were observed in fish exposed to all test media, except in the reference group (Fig. 6). In the Al-rich medium, the severity of damage increased with exposure time and with decreasing water residence time (Table 3). In chamber 1, the gills were strongly damaged after 2 days of exposure in the Al-rich medium (Fig. 6A). In chamber 2, the same degree of damage was apparent after 3 days (Table 3) but not after 2 days (Fig. 6B). In chamber 3, gills were not affected after 1 day (Table 3), but minor structural alterations were evident after 2 days (Fig. 6C). After 3 days, the gills showed clubbing of the filaments and some lamellar fusion in chamber 3. Fish gills exposed to the Al-poor medium showed no evidence of structural changes after 2 days (Fig. 6D).

Discussion

In the present study, we have shown that mortality and the extent of gill damage and physiological disturbances in fish exposed to a transient Al chemistry are dependent on the degree of ongoing Al polymerisation. Our results, however, neither support nor contradict the hypothesis that hypoxia is the primary physiological effect in fish exposed to such conditions. The present study also supports earlier indications

Fig. 5. (A) Blood haematocrit, (B) plasma lactate, (C) plasma Cl^-, and (D) plasma Na^+ in Arctic char exposed to the Al-rich medium (squares) and the Al-poor medium (control (circles)) (means ± SE, $n = 5$ if not stated otherwise on the graph). Water residence time: solid squares, 1 min; hatched squares, 3 min; open squares, 15 min. Open circles indicate the reference group sampled immediately before the exposures were started. An asterisk indicates a significant difference from the control (one-way ANOVA, Tukey–Kramer comparison, $p < 0.05$). Significant differences ($p < 0.05$) among Al treatments, i.e., water residence time, at one sampling period are indicated by treatment groups that do not share a common letter. If no letters are present for the sampling period, there were no significant differences between means.



that the Arctic char is a salmonid relatively tolerant to acidic Al-rich water.

Effects of a transient Al chemistry

Our results reveal that mortality in Arctic char corresponded well to water residence time, i.e., the degree of ongoing Al polymerisation. We also observed that the effects of Al on haematology and gill morphology as well as on mortality depended on the degree of Al polymerisation. It has been documented in a number of studies that a transient Al chemistry may dictate the Al toxicity to fish in acidified freshwater systems (e.g., Poléo et al. 1994; Witters et al. 1996). In our experiments, the effects of Al decreased systematically with water residence time through the experimental system, and the decrease corresponded to a systematic decrease in Al_i, i.e., positively charged low molecular weight Al species. Concurrently with this, we observed an increase in nonreactive high molecular weight Al_c. These observations confirm that Al toxicity decreases as the Al polymers grow (age) and their net charge approaches zero (Poléo et al.

Table 4. Comparison of relative toxicity in salmonids exposed to Al under non-steady-state conditions.

Species	LT_{50} (h)	Al ($mg \cdot L^{-1}$)	pH	Ca ($mg \cdot L^{-1}$)	Total organic C ($mg\ C \cdot L^{-1}$)	Temperature (°C)	Reference
Brown trout	7	<250	5.8	2.3		16–22	Rosseland et al. 1992
Brown trout	7	300	6.2	2.5	1.5	9.5	Lydersen et al. 1994
Brown trout	24	<100	6.4	25.0		16.0	Witters et al. 1996
Atlantic salmon	7	<250	5.8	2.3		16–22	Rosseland et al. 1992
Atlantic salmon	60	250	5.6	1.8	2.3	7.0	Poléo et al. 1994
Arctic char	65	480	5.8	2.9	3.0	7.5	This study

1994; Poléo 1995). Therefore, the acute toxicity of Al to fish seems to be a matter of reaction kinetics (Lydersen et al. 1991; Exley et al. 1996) where the degree of ongoing Al polymerisation is the most important factor (Poléo 1995). In support of this, several authors have documented fish mortality immediately after a rise in pH (e.g., liming) or temperature (e.g., Grahn 1980; Baker and Schofield 1982). In our experiments, the pH of the $Al(NO_3)_3$ solution was raised from 2.0 to 5.8 in the experimental channel.

The external surface of fish gills (mucus and epithelia) is rich in negatively charged sites (Wold and Selset 1977). Positively charged Al species may therefore easily bind to the gill surface (Wilkinson and Campbell 1993). We observed that the amount of Al accumulated at the gill surface was negatively correlated with the water residence time, i.e., a decrease in net positive charge of the Al species. Poléo (1995) suggested that the negatively charged sites at the gill surface act as Al polymerisation nuclei and that further growth and ageing of Al particles take place on the gill surface subsequent to the binding of positively charged low molecular Al species. Playle and Wood (1990) found that about 35% of Al was removed from solution if the pH was raised from 5.3 to 5.7. They concluded that the production of high molecular weight Al species was fast enough to explain Al accumulation on fish gills partly by polymerisation and partly by precipitation. Since the total amount of Al in our experimental water did not change with the residence time, it is most likely that Al accumulates at the gill surface due to the process of binding and subsequent Al polymerisation (Poléo 1995) rather than to precipitation of $Al(OH)_3$. In accordance with this, it has been demonstrated, by use of an Al_{26}-tracer, that Al bound to fish gills following a change in pH arose from polymerisation of low molecular weight Al species (Oughton et al. 1992). Exley et al. (1996) showed that Al precipitated at the gill surface was not always associated with an acute toxicity. Instead, they proposed that the strength of association of Al with the gill surface was the determining factor for the acute toxicity. It has also been suggested that the formation of the ${Al_{13}}^{7+}$ polymer in the gill microenvironment is responsible for the acute toxicity (Bertsch 1987; Bertsch and Parker 1996). On the other hand, there is no evidence anywhere in the literature that this polynuclear form could form under the non-steady-state conditions used in our experiments (Gensemer and Playle 1999). If it did form, however, it would probably not be stable for a long enough time to exert any toxicity (Furrer et al. 1992). Added to this fact is that the only experiment that directly compared the toxicities of Al^{3+} and ${Al_{13}}^{7+}$ in a steady-state system showed no significant toxicity for the polymeric form (Exley et al. 1994). Finally, Exley et al. (1996) suggested that the acute toxicity evoked under non-steady-state conditions was due to the diffusion of small Al polymers into the mucus matrix, which resulted in tanning of mucus by Al cross-linking the mucus glycocalyx. Poléo (1995), on the other hand, hypothesised that the toxicity is due to accumulation of Al by growth and ageing of Al particles on the gill surface subsequent to the binding of positively charged low molecular Al species, in addition to increased mucus secretion.

The biological importance of unstable mixing zones in nature, in which Al polymerisation takes place, has been the subject of debate (Rosseland et al. 1992; Lydersen et al. 1994; Åtland and Barlaup 1995). Based on the relatively rapid decrease in toxicity along our experimental channel, we believe that the extent of toxic mixing zones in nature will be limited. The Al toxicity in non-steady-state systems, however, will depend on the concentrations of inorganic Al species, water temperature, and the relative change in pH.

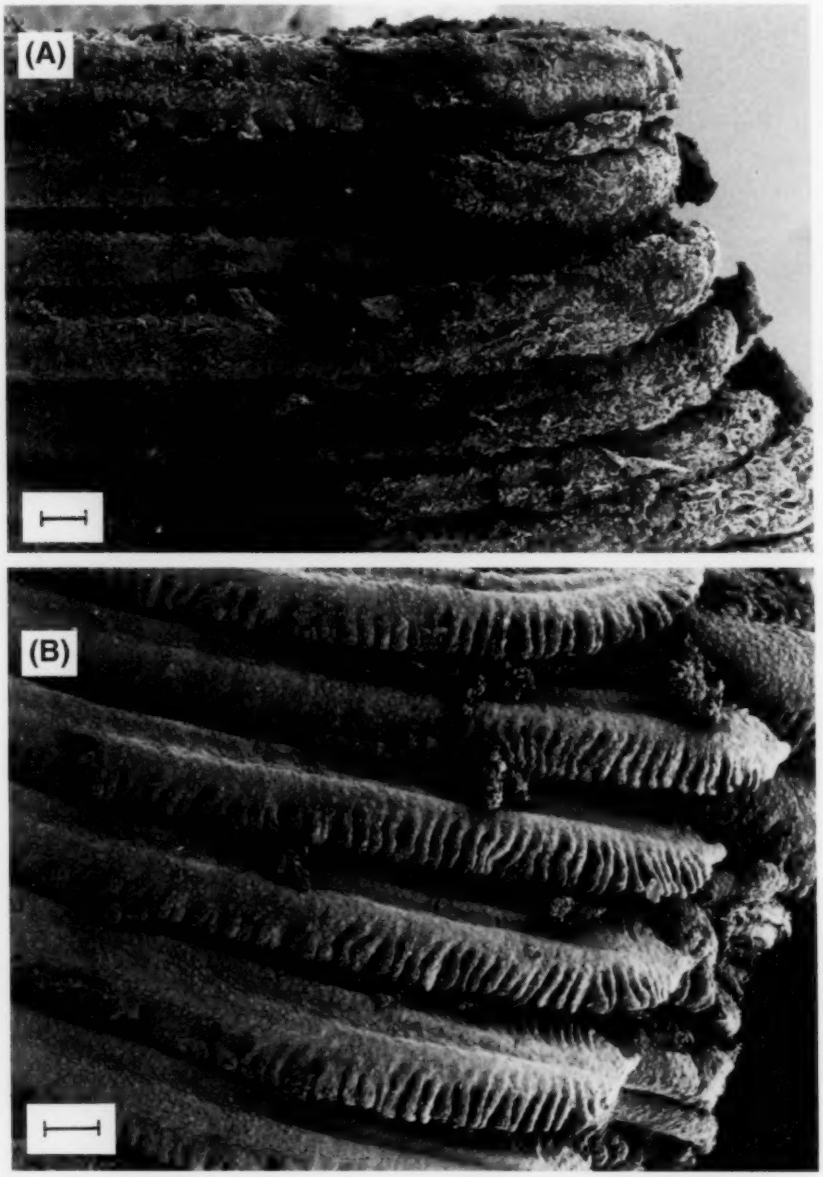
Mechanism of acute Al toxicity in fish

The consequences of Al exposure in fish appear to be respiratory and ion regulatory disturbances (e.g., Neville 1985; Gensemer and Playle 1999). Typical symptoms are plasma acidosis, hypoxia and hypercapnia, loss of electrolytes (especially Na^+ and Cl^-), increased blood haematocrit, and reduced plasma osmolality. Several authors have also observed inflammation (oedemas and swelling), and in some cases lamellar fusion, in the gills of Al-exposed fish (e.g., Schofield and Trojnar 1980; Tietge et al. 1988). Furthermore, it has very often been reported that fish exposed to acidic Al-rich water have abnormal amounts of mucus on their gills (e.g., Schofield and Trojnar 1980; Dietrich and Schlatter 1989).

A major problem, however, has been in deciding which of these biological symptoms are primary effects and which symptoms are secondary effects of Al. Neville (1985) demonstrated that hypoxia was an important factor in the mortality of rainbow trout (*Oncorhynchus mykiss*) at relatively high pH (5.5–6.5), i.e., when conditions for Al polymerisation and accumulation are optimal (Hem and Roberson 1967; Lydersen et al. 1991). Accordingly, both Poléo (1995) and Exley et al. (1996) hypothesised that acute hypoxia is the primary effect of Al toxicity in fish under non-steady-state conditions. Observations made by Witters et al. (1996) support this hypothesis. They reported that both haematocrit and ventilation frequency increased in brown trout (*Salmo trutta*) exposed to Al under non-steady-state conditions and that the effects were correlated with the degree of Al polymerisation.

In the present study, Arctic char also seemed to suffer from hypoxia. Plasma lactate increased, and according to the scanning electron micrographs, the total gill surface area seemed to decrease during the Al exposure. Furthermore,

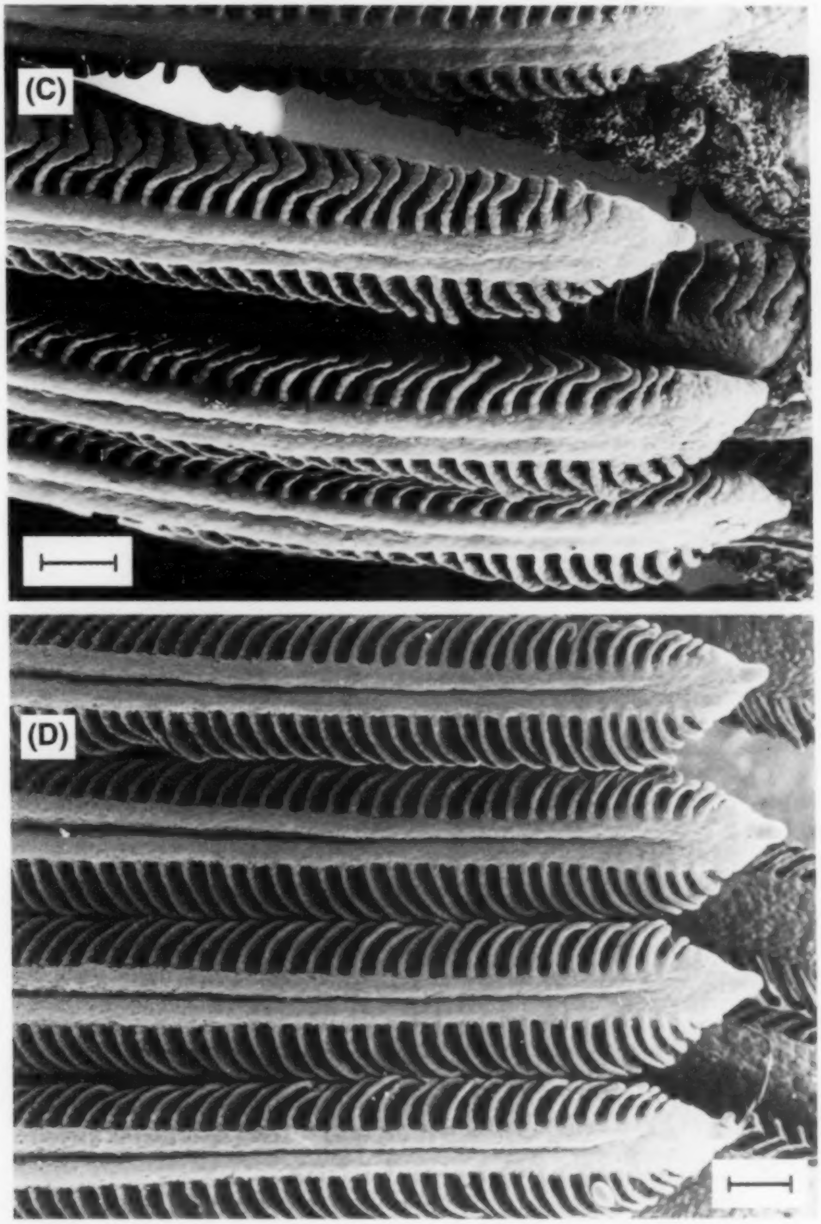
Fig. 6. Scanning electron micrographs of gill filaments of Arctic char after 2 days in the Al-rich medium. (A) Epithelial desquamation and general loss of gill structure in fish from chamber 1, with severe hyperplasia and fusion of lamellae along with extensive amounts of debris. (B) Extensive clubbing and some lamellar fusion in fish from chamber 2. (C) No cellular proliferation but some clubbing of the lamellae from fish in chamber 3. (D) There was no evidence of gill lesions in fish from the Al-poor medium. Magnification = 100×; scale bars = 100 μm.



these effects were more severe in the initial phase of the Al polymerisation process and decreased along with the decrease in net positive charge of the Al species present in solution. A possible conclusion that hypoxia is the primary effect of Al is also supported by the observed high resistance to acidic Al-rich water in the brown bullhead (*Ameiurus nebulosus*, previously *Ictalurus nebulosus*) (Bogaerts et al. 1991) and the crucian carp (*Carassius carassius*) (Poléo et al. 1995). The brown bullhead has a relatively high tolerance for environmental hypoxia (Beamish 1964), while the crucian carp is one of the most anoxia-tolerant vertebrates known (Nilsson et al. 1991).

In our work and in the report by Witters et al. (1996), however, both brown trout and Arctic char showed marked symptoms of ionoregulatory dysfunction, and these disturbances appeared simultaneously with the symptoms of

Fig. 6 (*concluded*).



hypoxia. We therefore suggest that the primary effect of Al under non-steady-state conditions is structural alterations of the gill epithelia, such as those revealed by the scanning electron micrographs, and that polymerisation and accumulation of Al on the gill surface probably cause these alterations. Furthermore, we suggest that both hypoxia and ionoregulatory dysfunction are secondary effects subsequent to the epithelial lesions. This is supported by the rapid decline in all haematological responses that we observed along the experimental channel and the fact that these followed precisely the declining degree of gill lesions. It is possible, however, that cellular or epithelial hypoxia can precede the gill lesions, systemic hypoxia, and ionoregulatory dysfunction observed by Witters et al. (1996) and ourselves.

Al toxicity in Arctic char

In a previous work, the Arctic char has been characterised as a freshwater fish species relatively tolerant to Al (Poléo et al. 1997). Moreover, if we compare our results with what has been documented concerning Al toxicity in other salmonid species under non-steady-state conditions (Table 4), the Arctic char appears to be relatively tolerant to Al (Rosseland et al. 1992; Lydersen et al. 1994; Poléo et al. 1994; Witters et al. 1996). It was reported that Atlantic salmon (*Salmo salar*) parr had approximately the same LT_{50} (60 h) (Poléo et al. 1994) as the Arctic char in the present study (65 h in chamber 1). In the experiment performed by Poléo et al. (1994), however, the total concentration of Al was 250 $\mu g \cdot L^{-1}$ compared with 480 $\mu g \cdot L^{-1}$ in our experiment. Thus, the relative toxicity of Al between salmonids in the studies under non-steady-state conditions confirms the previous observations made by Poléo et al. (1997) under more stable conditions, i.e., that the Arctic char is among the more Al-tolerant salmonid species. In this regard, it is also interesting to note that the Arctic char has been characterised as a relatively hypoxia-tolerant salmonid (Baroudy and Elliott 1994). Thus, the relative high tolerance to Al observed in the Arctic char can probably be explained by its relatively high tolerance to low oxygen levels.

Poléo et al. (1997) reported 35% mortality in Arctic char after 250 h of exposure to Al. After this initial mortality, only two individuals out of the remaining 54 fish died during the next 230 h of the experiment. We found that the same tendency was evident in the present study. Except from the first chamber in channel A, we observed that mortality ceased after the initial mortality in chambers 2 and 3. In addition to this, it also seemed to be that the physiological disturbances in the fish ceased to some extent towards the end of the physiological experiment, even though this tendency was not statistically significant. Interestingly, McDonald et al. (1991) observed such physiological changes in a species closely related to the Arctic char, the brook trout (*Salvelinus fontinalis*), exposed to sublethal Al at pH 5.2. Our observations suggest either that Arctic char has an ability to acclimate to Al-rich water or that the sensitivity to Al differs strongly in the population. Along with a possible physiological recovery, we also observed a decrease (not statistically significant) in the amount of Al accumulated on the gills towards the end of the exposures. This might indicate that the fish acclimate to the Al exposure. Indeed, acclimation to Al stress in brook trout has been shown and discussed previously by several authors (e.g., Wood et al. 1988; McDonald et al. 1991). Accordingly, McDonald et al. (1991) observed that it was a progressive reduction of gill Al in brook trout after an initial accumulation when exposed to sublethal Al. They concluded that the fish acclimated to the Al challenge and that the acclimation process resulted from specific changes at the gills. Such changes might be an increased rate of mucus production and sloughing, according to Wilkinson and Campbell (1993). As far as we know, however, it has never before been reported that fish can acclimate to Al during exposures to high concentrations as well as under non-steady-state chemical conditions such as those used in the present study. We exposed the fish to 480 $\mu g\ Al \cdot L^{-1}$ in the present study compared with 75 $\mu g\ Al \cdot L^{-1}$ in the study by McDonald et al. (1991). Lacroix et al. (1993), on the other hand, studied Al deposition on gills in Atlantic salmon fry and found that the amount of Al on the gills increased during the entire exposure. After transfer to optimal water quality conditions, however, the Al deposited on the gills was rapidly eliminated (Lacroix et al. 1993). This was in accordance with observations made by Wilkinson and Campbell (1993), also studying Atlantic salmon. The Atlantic salmon has been documented to be one of the most Al-sensitive freshwater fish species studied, in contrast with the brook trout and the Arctic char (Poléo et al. 1997). This might be the explanation of why no acclimation has been observed in studies with the Atlantic salmon. Further investigations, however, are needed to confirm the mechanism behind the observed resistance to high concentrations of Al in surviving individuals of Arctic char.

Acknowledgements

Special thanks to Dr. Chris Exley, Keele University, U.K., and to Professor Per Enger, Professor Asbjørn Vøllestad, and Joachim Schjolden, all at the Department of Biology, University of Oslo, for constructive criticism and help during the preparation of the manuscript. We also thank two anonymous referees for their comments that significantly improved the quality of our manuscript.

References

Åtland, Å., and Barlaup, B.T. 1995. Avoidance of toxic mixing zones by Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) in the limed River Audna, southern Norway. Environ. Pollut. **90**: 203–208.

Baker, J.P., and Schofield, C.L. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. **18**: 289–309.

Barnes, R.B. 1975. The determination of specific forms of aluminium in natural water. Chem. Geol. **15**: 177–191.

Baroudy, E., and Elliott, J.M. 1994. Tolerance of parr of Arctic charr, *Salvelinus alpinus*, to reduced dissolved oxygen concentrations. J. Fish Biol. **44**: 736–738.

Beamish, F.W.H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. Can. J. Zool. **42**: 177–188.

Bertsch, P.M. 1987. Conditions for Al_{13} polymer formation in partially neutralized aluminum solutions. Soil Sci. Soc. Am. J. **51**: 825–828.

Bertsch, P.M., and Parker, D.R. 1996. Aqueous polynuclear aluminum species. *In* The environmental chemistry of aluminum. 2nd ed. *Edited by* G. Sposito. Lewis Publishers, New York. pp. 117–168.

Bogaerts, K.M., Witters, H.E., Vets, S., and Vanderborght, O.L.J. 1991. Aluminium and pH effects on some osmoregulatory and haematological parameters of the acid-resistant American bullhead *Ictalurus nebulosus* (Le Sueur). Belg. J. Zool. **121**: 81–86.

Cronan, C.S., and Schofield, C.L. 1979. Aluminum leaching response to acid precipitation: effects on high-elevation watersheds in the northeast. Science (Washington, D.C.), **204**: 304–306.

Dietrich, D., and Schlatter, C. 1989. Aluminium toxicity to rainbow trout at low pH. Aquat. Toxicol. **15**: 197–212.

Driscoll, C.T. 1984. A procedure for the fractionation of aqueous aluminium in dilute acidic water. Int. J. Environ. Anal. Chem. **16**: 267–283.

Driscoll, C.T., and Postek, K.M. 1996. The chemistry of aluminum in surface waters. *In* The environmental chemistry of aluminum. 2nd ed. *Edited by* G. Sposito. Lewis Publishers, New York. pp. 363–418.

Exley, C., Wicks, A.J., Hubert, R.B., and Birchall, J.D. 1994. Polynuclear aluminium and acute toxicity in the fish. J. Theor. Biol. **167**: 415–416.

Exley, C., Wicks, A.J., Hubert, R.B., and Birchall, J.D. 1996. Kinetic constraints in acute aluminium toxicity in the rainbow trout (*Oncorhynchus mykiss*). J. Theor. Biol. **179**: 25–31.

Furrer, G., Trusch, B., and Müller, C. 1992. The formation of polynuclear Al_{13} under simulated natural conditions. Geochim. Cosmochim. Acta, **56**: 3831–3838.

Gensemer, R.W., and Playle, R.C. 1999. The bioavailability and toxicity of aluminum in aquatic environments. Crit. Rev. Environ. Sci. Technol. **29**: 315–450.

Grahn, O. 1980. Fishkills in two moderately acid lakes due to high aluminum concentration. *In* Ecological impact of acid precipitation. *Edited by* D. Drabløs and A. Tollan. SNSF-project. Proc. Int. Conf., Sandefjord, Norway. pp. 310–311.

Hem, J.D., and Roberson, C.E. 1967. Form and stability of aluminum hydroxide complexes in dilute solutions. U.S. Geol. Surv. Water-Suppl. Pap. 1827-A.

Lacroix, G.L., Peterson, R.H., Belfry, C.S., and Martin-Robichaud, D.J. 1993. Aluminum dynamics on gills of Atlantic salmon fry in the presence of citrate and effects in integrity of gill structures. Aquat. Toxicol. **27**: 373–402.

Lydersen, E., Salbu, B., and Poléo, A.B.S. 1991. Formation and dissolution kinetics of $Al(OH)_3$ (s) in synthetic freshwater solutions. Water Resour. Res. **27**: 351–357.

Lydersen, E., Poléo, A.B.S., Nandrup Pettersen, M., Riise, G., Salbu, B., Kroglund, F., and Rosseland, B.O. 1994. The importance of "in situ" measurements to relate toxicity and chemistry in dynamic aluminium freshwater systems. J. Ecol. Chem. **3**: 357–365.

McDonald, D.G., Wood, C.M., Rhem, R.G., Mueller, M.E., Mount, D.R., and Bergman, H.L. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). I. Physiology. Can. J. Fish. Aquat. Sci. **48**: 2006–2015.

Neville, C.M. 1985. Physiological responses of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum — prediction of field responses from laboratory data. Can. J. Fish. Aquat. Sci. **42**: 2004–2019.

Nilsson, G.E., Lutz, P.L., and Jackson, T.L. 1991. Neurotransmitters and anoxic survival of the brain: a comparison of anoxia-tolerant and anoxia-intolerant vertebrates. Physiol. Zool. **64**: 638–652.

Oughton, D.H., Salbu, B., and Bjørnstad, H.E. 1992. Use of an aluminium-26 tracer to study the deposition of aluminium species on fish gills following mixing of limed and acidic waters. Analyst, **117**: 619–621.

Playle, R.C., and Wood, C.M. 1990. Is precipitation of aluminum fast enough to explain aluminum deposition on fish gills? Can. J. Fish. Aquat. Sci. **47**: 1558–1561.

Poléo, A.B.S. 1995. Aluminium polymerisation — a mechanism of acute toxicity of aqueous aluminium to fish. Aquat. Toxicol. **31**: 347–356.

Poléo, A.B.S., Lydersen, E., Rosseland, B.O., Kroglund, F., Salbu, B., Vogt, R.D., and Kvellestad, A. 1994. Increased mortality of fish due to changing Al-chemistry of mixing zones between limed streams and acidic tributaries. Water Air Soil Pollut. **75**: 339–351.

Poléo, A.B.S., Øxnevad, S.A., Østbye, K., Andersen, R.A., Oughton, D.H., and Vøllestad, L.A. 1995. Survival of crucian carp, *Carassius carassius*, exposed to a high low-molecular weight inorganic aluminium challenge. Aquat. Sci. **57**: 350–359.

Poléo, A.B.S., Østbye, K., Øxnevad, S.A., Andersen, R.A., Heibo, E., and Vøllestad, L.A. 1997. Toxicity of acid aluminium-rich water to seven freshwater fish species: a comparative laboratory study. Environ. Pollut. **96**: 129–139.

Rosseland, B.O., Blakar, I.A., Bulger, A., Kroglund, F., Kvellestad, A., Lydersen, E., Ougthon, D.H., Salbu, B., Staurnes, M., and Vogt, R.D. 1992. The mixing zone between limed and acidic river waters: complex aluminium chemistry and extreme toxicity for salmonids. Environ. Pollut. **78**: 3–8.

Schofield, C.L., and Trojnar, J.R. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters. *In* Polluted rain. *Edited by* T.Y. Toribara, M.W. Miller, and P.E. Morrow. Plenum Press, New York. pp. 341–362.

Sparling, D.W., and Lowe, T.P. 1996. Environmental hazards of aluminum to plants, invertebrates, fish, and wildlife. Rev. Environ. Contam. Toxicol. **145**: 1–127.

Tietge, J.E., Johnson, R.D., and Bergman, H.L. 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum. Can. J. Fish. Aquat. Sci. **45**: 1643–1648.

Wilkinson, K.J., and Campbell, P.G.C. 1993. Aluminum bioconcentration at the gill surface of juvenile Atlantic salmon in acidic media. Environ. Toxicol. Chem. **12**: 2083–2095.

Witters, H.E., Van Puymbroeck, S., Stouthart, A.J.H.X., and Wendelaar Bonga, S.E. 1996. Physico-chemical changes of aluminium in mixing zones: mortality and physiological disturbances in brown trout (*Salmo trutta* L.). Environ. Toxicol. Chem. **15**: 986–996.

Wold, J.K., and Selset, R. 1977. Glycoproteins in the skin mucus of the char (*Salmo alpinus* L.). Comp. Biochem. Physiol. B, Comp. Biochem. **56**: 215–218.

Wood, C.M., McDonald, D.G., Booth, C.E., Simons, B.P., Ingersoll, C.G., and Bergman, H.L. 1988. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. **45**: 1587–1596.

Comparisons within and between years resulting in contrasting recruitment of Pacific hake (*Merluccius productus*) in the California Current System

Michael M. Mullin, Erica Goetze, Stace E. Beaulieu, and Jacqueline M. Lasker

Abstract: In a search for correlates and possible causes of the difference in recruitment success of Pacific hake (*Merluccius productus*) of the 1981 and 1984 year-classes, we analyzed (by optical plankton counter) the distribution into sizes of preserved zooplankton samples from stations with and without larval hake in winter and spring of these two years. We also assessed the onshore/offshore distributions of larvae, potential for geostrophic and Ekman transport, and their overall survival rates. Cluster analyses of biovolume spectra led to the development of a simple ratio of "medium" to "small + large" zooplankton that statistically separated stations within a year where larval hake were likely to be found from those where they were unlikely to be found. This biovolume ratio was independent of temperature, and the same ratio statistically distinguished stations where larval hake tended to occur in spring 1998. However, we found no property or process that might explain why the 1984 year-class was spectacularly successful.

Résumé : Dans la recherche de corrélats et de causes possibles de la différence dans le succès de recrutement du merlu du Pacifique des classes 1981 et 1984, nous avons analysé (au compteur optique à plancton) la répartition des tailles dans des échantillons conservés de zooplancton provenant de stations avec ou sans présence de larves de merlu, à l'hiver et au printemps de ces deux années. Nous avons aussi évalué les répartitions côtières et hauturières des larves, le potentiel de transport géostrophique et de transport d'Ekman, ainsi que les taux globaux de survie. Les analyses typologiques des spectres du biovolume ont permis d'établir un rapport simple du zooplancton de taille moyenne au zooplancton de taille petite + grande, qui permettait de séparer statistiquement les stations où les larves de merlu étaient susceptibles d'être présentes au cours d'une année et les stations où elles ne l'étaient pas. Ce rapport du biovolume était indépendant de la température, et le même rapport a permis de repérer statistiquement les stations où les larves de merlu étaient présentes au printemps de 1998. Toutefois, nous n'avons découvert aucune propriété ni aucun mécanisme qui puisse expliquer pourquoi la classe 1984 a présenté un succès spectaculaire.

[Traduit par la Rédaction]

Introduction

Pacific hake (*Merluccius productus*) (also called Pacific whiting) is an important commercial species that lives within the California Current System (CCS), spawning primarily south of Monterey but fished primarily off Oregon, Washington, and British Columbia (Bailey et al. 1982; Francis 1983). There are congeneric stocks of commercial importance in other eastern boundary current systems (e.g., Alheit and Pitcher 1995). From the U.S. west coast fishery, which developed to large scale in the late 1960s, it is known that interannual variability in recruitment of hake is very large; strong year-classes, occurring several years apart, sustain the fishery for several years.

Specifically, a time series of parental abundance (as spawning biomass) and subsequent recruitment (as 2-year-old fish) of year-classes from 1972 to 1993 (Dorn 1996) (Fig. 1) shows that the 1981 year-class consisted of 0.12×10^9 recruits from a spawning biomass of 1.4×10^6 tons, while in 1984, a similar spawning biomass (1.6×10^6 tons) gave rise to 9.8×10^9 recruits, the second largest recruitment in Dorn's (1996) 24-year record. We examined environmental data from these two years for possible correlates of recruitment success because field sampling during those years was done on a sufficiently large scale to include most of the hake's spawning range.

Natural history of larvae

Hake larvae are most frequently encountered, and are most abundant, in winter and spring (Moser et al. 1993). They live deeper than do the larvae of most well-studied species (Ahlstrom 1959; Mullin and Cass-Calay 1997). The larvae are zooplanktivorous (Sumida and Moser 1980), and at least in some years, larval growth (Cass-Calay 1997) and survival (Butler and Nishimoto 1997) are food limited. How-

Received October 22, 1999. Accepted March 10, 2000.
J15416

M.M. Mullin,[1] E. Goetze, S.E. Beaulieu,[2] and J.M. Lasker. Marine Life Research Group, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0227, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: mmullin@ucsd.edu

[2] Present address: Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, U.S.A.

ever, it is far from clear that food limitation is a dominant determinant of subsequent recruitment.

Interannual variation in temperature

The winter of 1984 was affected by the waning of a major Californian El Niño along the North American west coast. Bailey and Francis (1985, their fig. 5) had reported that years of strong recruitment by hake were anomalously warm in the CCS (although not all warm years resulted in strong recruitment), and Hollowed et al. (1998) recognized that these years of strong recruitment came during Californian El Niño events. Bailey (1981, his fig. 10) also reported a positive relationship between larval survival (a proxy based on the ratio of large to small larvae in a year-class) and temperature in the Southern California Bight in the 1960s.

However, the causal mechanism linking good survival and occasional strong recruitment to the anomalous warmth (and other, less well-documented environmental changes) is unclear. Indeed, the relationship of strong recruitment to Californian El Niño events complicates the issue of whether recruitment is food limited, since the biomass of macrozooplankton in the CCS decreases during El Niños (Chelton et al. 1982).

Bailey (1980), Hollowed (1992), and others have noted that main spawning areas vary interannually, depending in part on the sizes of the parents (large adults spawn farther south than do small ones) and climate (spawning occurs farther south in cold years: Bailey 1980, his fig. 2; Bailey and Francis 1985). Hollowed (1992) reported that in 1984, but not in 1981, larval hake were found north of the region sampled by the California Cooperative Oceanic Fisheries Investigations (CalCOFI) (Fig. 2) as well as within it. On a large time scale, Horne and Smith (1997) showed a 35-year trend towards more northerly centers of larval abundance, paralleling a warming trend in the CCS (Roemmich 1992). However, they note that, in spite of these correlations, temperature is not a good indicator of annual recruitment, since several warm years tend to occur sequentially, but strong recruitment of hake is a single event, usually followed by poor years. Nor is it obvious from Fig. 1 that strong year-classes have become either more frequent or stronger as the CCS has warmed.

Interannual variation in transport

Temperature is a proxy for other aspects of the physical environment, in that some of the relationship between recruitment and temperature probably reflects physical processes rather than temperature itself, such as transport of larvae to areas suitable for juvenile life. For hake, these areas are the shelf <200 m deep, since this is where the youngest juveniles are found (Bailey 1981; Bailey et al. 1982). Postspawning advection of eggs and larvae may vary interannually, offshore advection due to wind-driven coastal upwelling (cool surface water) versus onshore advection during downwelling (warm surface water) being the most frequently discussed mechanism. Strong year-classes have been associated with years either of weak upwelling or of downwelling (Bailey and Francis 1985), although not all year-classes in such years are strong. We therefore examined the onshore/offshore position of larvae, and indices of upwelling, in 1981 and 1984.

Bailey (1980) argued that this source of variation was particularly important in central California and less so in Baja California. Although the larval hake live below the Ekman layer most affected by this process, the center of mass of their distribution off central California is farther offshore during years of strong upwelling (Bailey 1980), perhaps because eggs float into the Ekman layer after being spawned and the larvae descend after hatching. However, Bailey (1981) added that, because of the depth at which larvae occurred, they might actually be carried shoreward during periods of weak upwelling, as surface water moving offshore is replaced by deeper water. Critical to this line of argument is precise description of the movement of water in terms of distances from the coast and depth-stratified velocities. For example, if most of the subsurface water moving shoreward during wind-driven upwelling is actually confined to the benthic boundary layer of the shelf, it is unlikely that larval hake would be transported in this flow.

Another possible mechanism, which we did not test, is that anomalously strong poleward flow over the shelf, known to occur during some Californian El Niños, promotes the transport of young-of-the-year juveniles to northern feeding grounds. The causal mechanism for promotion of recruitment would therefore be advection acting on the postlarval phase.

Present study

Our primary purpose in this study was to determine whether the macrozooplankton of the spawning seasons of 1981 and 1984 provided any clues as to the marked difference between these years in success of subsequent recruitment (considering the macrozooplankton more as an environmental indicator than as a direct source of food for larval hake). We also examined other putative causal mechanisms, including differences between the two years in onshore/offshore location and transport of larvae, and we used proxies to assess the relative survival of larvae in 1981 and 1984. This is not to say that all occasions of strong recruitment result from the same causal mechanism or that strong recruitment in one year depends on only one casual mechanism; the instances of strong recruitment are so rare and so striking (Fig. 1) that they may result from sequential alignment of "favorable states" in several components of the environment of larvae (and of juveniles).

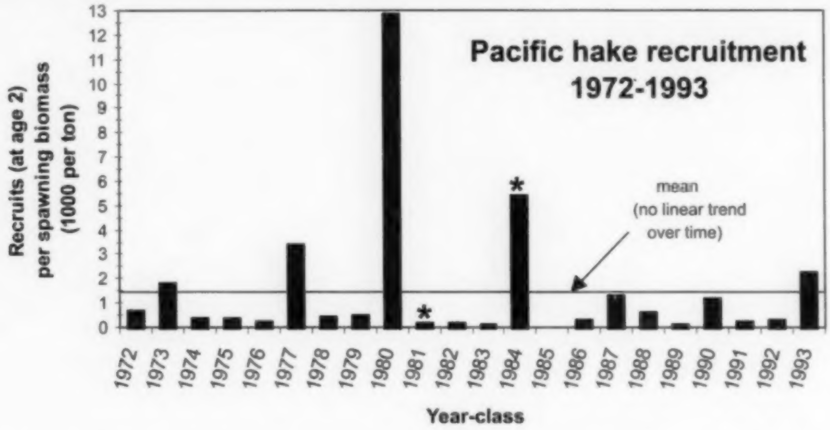
Finally, we determined whether there were detectable differences in the biomass or size distribution of macrozooplankton within years between locations where larval hake were found and those where they were not. Clarification of these issues would permit predictions of occurrence of larval hake or (more important for fisheries) their success of later recruitment.

Materials and methods

Available samples and data

During the 1970s and early 1980s, the CalCOFI monitoring program was sufficiently extensive to sample much of the spawning habitat of Pacific hake but was conducted only every third year. The regions sampled in the months of interest in 1981 and 1984 are shown in Figs. 2 and 3, together with the abundances of larval hake as enumerated by personnel of the Southwest Fisheries Sci-

Fig. 1. Pacific hake recruitment (thousands of age-2 fish) by year-class per spawning biomass in the year of birth (data from Dorn 1996). Asterisks indicate the two years compared in this study.



ence Center, U.S. National Marine Fisheries Service (NMFS); data are available in tabular form as National Oceanic and Atmoshpheric Organization (NOAA) Technical Memoranda (Ambrose et al. 1988; Stevens et al. 1990). Note that the locations where larvae were found result from the combined effects of spawning sites and egg/larval survival and advection since spawning.

The zooplankton samples were collected by towing a bridleless net of 505-μm mesh from the surface to 210 m or near bottom. For the purpose of this study, these samples have two chief weaknesses: (*i*) they were not depth stratified and thus include zooplankton from above the depths at which larval hake typically are most abundant and (*ii*) the mesh size was too large to retain quantitatively the sizes of zooplankton used by the youngest feeding larvae; the mouth width of a 3.3-mm larva is approximately 400 μm, although prey that large were actually found only in guts of larvae >4 mm in length and probably do not become a frequent part of the diet until a larva reaches 10 mm (Sumida and Moser 1980; also see Mullin and Cass-Calay 1997). On the other hand, some of the larger types of zooplankton caught could be predators on young larvae (Bailey and Yen 1983).

Samples were taken similarly in the spring of 1998. This permitted a test of conclusions, derived from the analyses of 1980s samples, concerning the relationships between zooplankton and the presence or absence of larval hake.

We arbitrarily established a boundary separating "nearshore" from "offshore" (Figs. 2*a* and 3*a*), roughly reflecting bottom topography. The bottom depths at the stations through which we drew this boundary ranged between 1 and 3 km. Vertical distributions of temperature, density (as σ_t), and dynamic height relative to 500 m at the stations in 1984 where samples were taken for larval hake are reported in SIO Refs. 84-18, 84-23, and 84-25 (Scripps Institution of Oceanography 1984*a*, 1984*b*, 1984*c*). From these distributions, we prepared a hydrographic section approximately paralleling the coast for each cruise and calculated onshore/offshore geostrophic flow in the 50- to 150-m layer, from dynamic heights at 50, 100, and 150 m relative to 500 m, at adjacent stations. In contrast with the time-averaged but more indirect proxies used by many previous investigators (see Introduction), these depictions of flow in 1984 are "snapshots" and may include relatively ephemeral features such as meanders of the California Current. Unfortunately, hydrographic measurements were made on only a few lines perpendicular to the coast in 1981, so analogous calculations would be much less meaningful. However, the static position of larvae relative to the inshore/offshore boundary is still relevant. For this same reason, we had to use temperature at 10 m (which was measured at all stations) as a proxy for the physical environment rather than that at a greater depth more directly relevant to the larvae.

Based on the hypotheses summarized above concerning the relationships between interannual variations in recruitment and in upwelling, we obtained from the Pacific Fisheries Environmental Laboratory, NMFS, NOAA (www.pfeg.noaa.gov/products/upwell.html), the monthly mean upwelling indices (calculated from winds derived from atmospheric pressure fields) and their anomalies from long-term means for January through May of 1981 and 1984 at 30°N, 119°W; 33°N, 119°W; 36°N, 122°W; and 39°N, 125°W. For each month and location, we took the difference between 1984 and 1981. We then weighted these differences to reflect the spatial and temporal distribution of larval hake, as indicated in Figs. 2 and 3 (e.g., February and March at 33°N, 119°W and 36°N, 122°W were given five times the weight of January and May at the northernmost and southernmost locations).

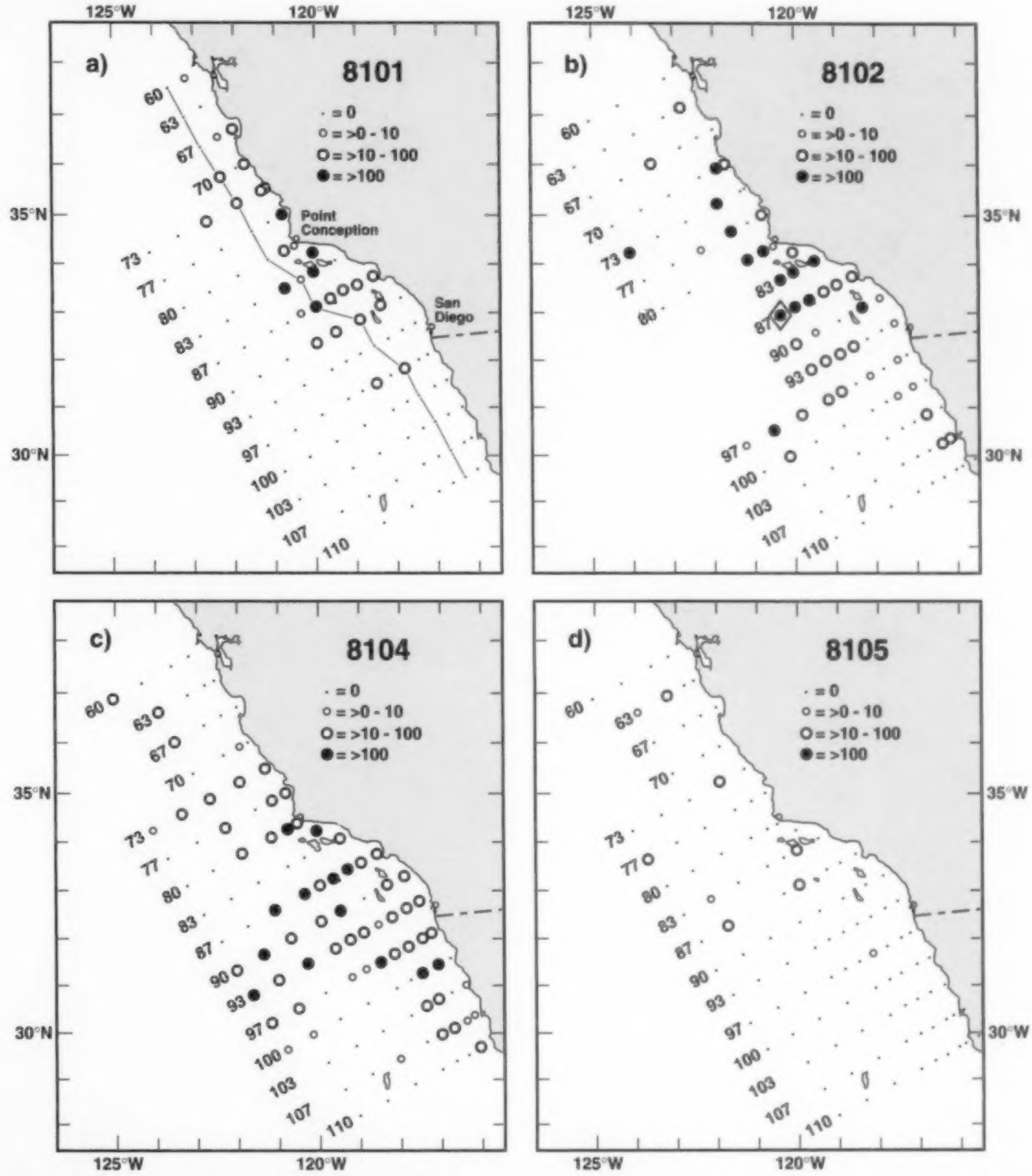
Optical plankton counter (OPC)

We used the OPC (described by Herman 1988) to analyze preserved samples of zooplankton. The device is designed to distinguish pulses (= individual particles) from turbidity in water flowing through a sensing zone across which a collimated beam of light shines on a photodiode and to measure the strength or height of the pulses. When a particle passes through the sensing zone, the OPC reports a digital size unit (DSU) that is proportional to the amount of light blocked by the particle, i.e., proportional to its silhouette. DSUs are converted to equivalent spherical diameters (ESD, the diameter of an opaque sphere that would give the same signal as that actually resulting from an irregularly shaped, possibly translucent, real animal at a particular orientation as it passes through the sensing zone) using a semiempirical, nonlinear equation determined by Herman (1992) from calibration with opaque spheres. An ESD can be further converted to volume through solid geometry. The design of our system for analyzing samples, and our calibrations with respect to actual zooplankters and to conventionally measured biovolumes of zooplankton in 1981 and 1984, are reported in Beaulieu et al. (1999).

To minimize the effects on conclusions of possible changes in the OPC or its operators, we alternated between the years in order of analysis of cruises, randomized the order of analysis of samples within each cruise, and concealed from the operators whether or not any samples contained larval hake.

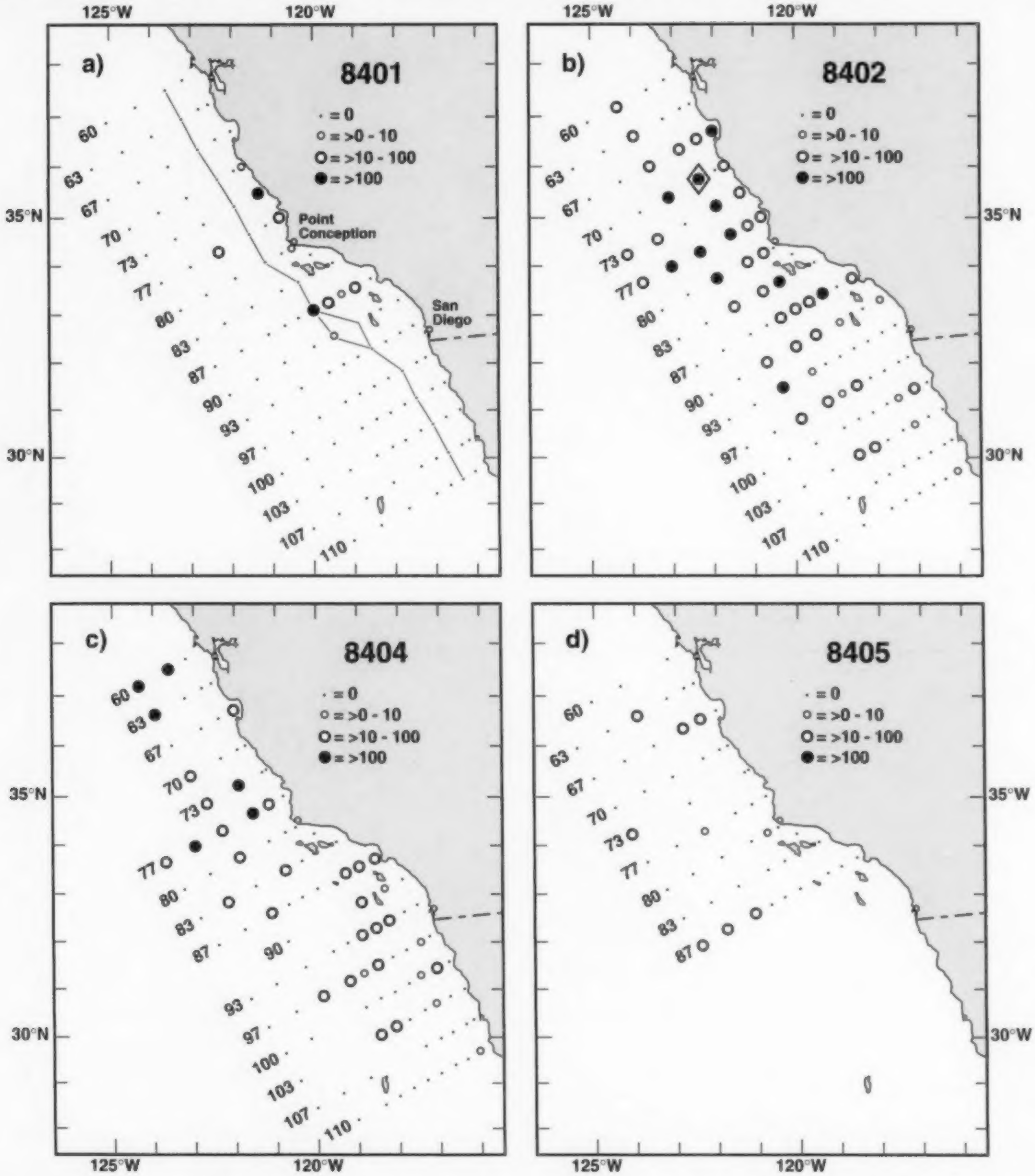
From the raw data reported by the OPC, we considered only those of DSU ≥ 50 (corresponding to an ESD ≥ 750 μm) as equiva-

Fig. 2. Stations sampled and abundances of larval Pacific hake (per 10 m^2) during winter–spring 1981: (*a*) January, (*b*) February, (*c*) April, and (*d*) May. The first two digits in a cruise number are the year and the last two digits are the month. The numbers along the offshore edge of the surveyed area are CalCOFI line numbers. The line approximately paralleling the coast in Fig. 2*a* shows the separation between "nearshore" and "offshore" larval occurrence. In Fig. 2*b*, the station enclosed in a diamond accounted for 49% of all larvae caught in 1981.



lent to cylindrical zooplankters that had been quantitatively retained by the net (i.e., an opaque cylinder of 2:1 length to diameter ratio and 470 μm in diameter would cast the same silhouette if passing through the OPC with the long axis parallel to the sensing surface) and minimized counting of small bubbles or detrital particles. We combined the raw data into 12 size categories of logarithmically (base 2) increasing width in DSU: 50, 50 + 2^0, 50 + 2^0 + 2^1, and so on. Although there are theoretical grounds for increasing the width of size categories of pelagic organisms logarithmically (references in Beaulieu et al. 1999), we chose this scheme primarily because real samples are likely to be dominated by small animals, so that significant changes in the numerical importance of large, rare animals might be difficult to detect. Combining the larger zooplankters of several sizes into a few wide categories accentuates the numerical importance of large animals but also smoothes minor differences in their OPC-sensed sizes

Fig. 3. Stations sampled and abundances of larval Pacific hake (per 10 m^2) during winter–spring 1984: (*a*) January, (*b*) February, (*c*) April, and (*d*) May. Hydrographic sections (Fig. 5) were constructed along the nearshore/offshore boundary (shown in Fig. 3*a*) for all four months. In Fig. 3*b*, the station enclosed in a diamond accounted for 66% of all larvae caught in 1984.



(which would vary depending on the orientations of individuals passing through the sensing zone). We then calculated the biovolume of each category as the number of counts per unit of water filtered times the volume of the appropriate sphere, calculated as the geometric mean ESD of the upper and lower boundaries of the category, and the total biovolume for each sample as the sum of biovolumes over all 12 categories.

We further combined data to consider, for example, biovolume of small zooplankters (arbitrarily defined as 750–860 μm ESD). These small zooplankters are larger than larval hake <1 cm in length commonly ingest, so are a poor surrogate for larval food, but are unlikely to be large enough to be predators on hake larvae. We also considered ratios of biovolumes of combined categories of zooplankters as indicators of the "shape" of the biovolume size spectrum. Initial analyses of size spectra of biovolumes suggested that the degree of upward convexity was an important distinction between samples. We therefore chose the following ratio, which increases with upward convexity, as a simple indicator of this property: (biovolume of zooplankters 1.16–2.70 mm ESD) × (biovolume 0.75–1.16 mm + biovolume >2.7 mm ESD)$^{-1}$.

Table 1. Hypotheses concerning environmental variables and larval Pacific hake in winter–spring 1981 and 1984.

Hypothesis code(s)	Statement(s)	Test(s)
1. Hypotheses concerning differences in zooplankton between stations within years		
$H_0 1a$	No significant differences between the median biovolumes or biovolume ratios at stations without larvae and at stations where larvae were found	Rank sum
$H_0 1Aa$	Explanatory zooplankton property is not correlated with 10-m temperature	Correlation
$H_0 1b$	No significant association between stations where larval hake were found and stations grouped according to their zooplankton	χ^2 or G
2. Hypotheses concerning differences in zooplankton between years		
$H_0 2a$	No significant differences between years in median biovolumes or biovolume ratios	Rank sum
$H_0 2b$ and $H_0 2c$	For only stations at which larvae occurred, no significant differences between years in medians Random assortment of these stations of the two years into clusters	Signed rank χ^2 or G
$H_0 2d$	For zooplanktonic assemblages with which larvae were most strongly associated, no significant difference in median biovolume ratios between years	Rank sum
3. Hypothesis concerning differences in larval location and transport between years		
$H_0 3a$	No significant difference between years in nearshore/offshore locations of larvae	Qualitative
$H_0 3b$	No significant difference in upwelling between years, weighted for larval abundances	Signed rank
4. Hypothesis concerning differences in larval survival between years		
$H_0 4$	No significant difference between years in larval survival	Inspection

Note: See text for elaboration of backgrounds of hypotheses.

Hypotheses and tests

A fundamental issue is whether abundance and composition of zooplankton are associated with recruitment success of hake: specifically, whether the macrozooplanktonic community present during the season of spawning and larval growth differed between 1981 and 1984 and could cause the enormous difference in recruitment success. In addition to the fact that the available samples are imperfect surrogates for actual food of larval hake (see above), there are several problems in approaching this fundamental question: (*i*) stations differ in important properties that might cause spatial or temporal variability in occurrence and success of larvae, other than the biomass and size distribution of zooplankton, (*ii*) the set of samples includes stations that are not even potential hake spawning sites (e.g., too shallow), (*iii*) sampling rare larvae: a positive occurrence certainly means that larvae were present at that station, but absence from the sample is not proof positive that no larvae experienced the conditions at that station and eventually recruited, (*iv*) the data are "snapshots" at specific places and times, while recruitment depends on an integration of events over the entire spawning and juvenile area and throughout prerecruit life, and (*v*) some issues, such as mortality due to piscivores, cannot even be approached with the available data.

If nonzooplankton properties of stations were independent of their zooplankton, the first problem could be addressed by using data on these other properties (e.g., temperature) in an analog of ANOVA. Both the total biovolume and the biovolume of small zooplankton were inversely correlated with 10-m temperature in both years ($p < 0.01$ in all cases) and hence could not be treated as variables independent of physical properties. However, the biovolume ratio described above was independent of 10-m temperature ($p > 0.10$ in both cases, for $N = 423$ in 1981 and $N = 395$ in 1984) and hence reflected a property of the zooplankton not linearly related to the physical environment. To address the second problem, we excluded from analysis all stations at which the net tow was shallower than 100 m (due to shallow bottom depth), although larvae were sometimes found in such samples.

We addressed the fundamental issue as a series of narrowly defined questions (each of which has a testable null hypothesis of no difference). Although we analyzed samples from May cruises, larval hake were rare then, and the 1984 cruise (8405) did not sample the southern half of the region (Figs. 2 and 3), so we tested most hypotheses using only data from January, February, and April.

(1) Within 1981 and (or) within 1984, did stations where larval hake were found differ in biovolume or size spectrum of zooplankton from those where larvae were absent?

We considered total biovolume, biovolume of the smaller zooplankton, and size spectrum as important parameters characterizing the macrozooplankton. One index of the size spectrum was the ratio of biovolumes described above. These questions are addressed as hypothesis $H_0 1a$ (Table 1).

(1A) If there were significant differences, were these in the properties of the zooplankton that were themselves correlated with the physical environment (hypothesis $H_0 1Aa$, Table 1)?

Such simple tests would be informative only if stations where larvae were found tended to be at one extreme or the other with respect to the property by which the stations were being ranked. We therefore addressed this question further by first establishing groupings of stations based on similarity of zooplankton and then determining whether stations where larval hake were found were nonrandomly associated with any particular groups (hypothesis $H_0 1b$, Table 1).

(2) Did 1984 differ from 1981 in biovolume or biovolume ratio of zooplankton (hypothesis $H_0 2a$, Table 1)?

Even if the years did not differ in zooplankton over the whole area and winter–spring period, the composition of macrozooplankton where larvae actually occurred could still indicate (or cause) different recruitment success. Therefore, we reframed questions of whether 1984 differed from 1981 to consider only those stations where larval hake occurred (hypotheses $H_0 2b$ and $H_0 2c$, Table 1).

Stated this way, the questions concern the stations where larval hake actually were caught, independent of the clusters in which these stations were grouped in dendrograms. Another approach (if $H_0 1b$ is rejected for both years) is to ask, did the zooplanktonic assemblages (i.e., clusters of stations) with which larval hake were most significantly associated in 1981 differ from those with significant associations in 1984? That is, we compare zooplanktonic assemblages with which larvae were significantly associated, even though larvae may not have been caught at all stations where the assemblage was found (hypothesis $H_0 2d$, Table 1).

The maps of geographical occurrences of larvae relative to a nearshore/offshore boundary are isolated representations of continuously changing pattern, and the paucity of hydrographic data in early 1981 prevented interannual comparison of advection into and out of the nearshore zone. Nevertheless, the following questions can be addressed.

Table 2. Median biovolumes and biovolume ratios (see text) for zooplankton from various groups of stations.

Year and stations	Total biovolume (mm^3/m^3)	Small zooplankton biovolume (mm^3/m^3)	Biovolume ratio	Null hypothesis
1981 with larvae	65.0**	2.4**	1.6**	H_01a
1981 without larvae	26.5**	0.97**	1.2**	
1984 with larvae	49.0**	2.1**	1.31**	H_01a
1984 without larvae	19.7**	0.87**	1.03**	
1981, all	49.2**	1.7**	1.38**	H_02a
1984, all	31.2**	1.3**	1.08**	
1981 with larvae	70.1	2.5	1.61**	H_02b
1984 with larvae	52.1	2.2	1.25**	
1981 larvae both years	78.3	2.7	1.63	H_02b
1984 larvae both years	79.9	2.5	1.39	
1981 zooplankton assemblage			1.92	H_02d
1984 zooplankton assemblage			1.63	

Note: Within-year comparisons were made only for January, February, and April; between-year comparisons were made for these months plus May. "With larvae" indicates those stations at which larval Pacific hake were caught in that year, "larvae both years" indicates those stations at which larvae occurred in the same month in both years, and "zooplankton assemblage" indicates the assemblage of zooplankton, from the cluster analyses, with which larval hake were most strongly associated. **Highly significant difference between the vertical pair ($p < 0.01$); *significant difference ($p < 0.05$). Null hypotheses are listed in Table 1.

(3) Did 1984 differ from 1981 in the occurrence of larvae in the nearshore zone (hypothesis H_03a, Table 1) or in monthly mean upwelling indices, weighted for the spatial/temporal distributions of larvae (hypothesis H_03b, Table 1)?

Because it is unclear what actual magnitudes of upwelling would be favorable or unfavorable for eventual recruitment, if H_03b is rejected, the interpretation of the acceptable alternative hypothesis is open to argument.

Bailey (1981) used the April abundance of larvae >10 mm long relative to the number of larvae spawned (estimated in January) as a measure of larval survival in the 1960s. Richard Charter of the Southwest Fisheries Science Center (NMFS, NOAA, personal communication) provided us with data on the lengths of larvae caught in winter–spring of 1981 and 1984, from which we calculated several proxies of larval survival so as to compare the two years. Unknown, but assumed to be similar in both years, is the distribution in time of sampling relative to that of spawning. If the magnitude of recruitment is determined in early larval life, one would expect larval survival to have been greater in 1984 than in 1981, whether availability of food, drift, or some other factor (e.g., predation) was the effective agent.

(4) Did 1984 differ from 1981 in larval survival (hypothesis H_04, Table 1)?

Size spectra and assemblages of zooplankton

As noted above, the OPC data permitted determination of zooplanktonic biovolume at each station as a function of various size categories — a biovolume spectrum — and stations could then be compared with respect to total biovolume, biovolume of a particular size category, or ratio of biovolumes of size categories (a simple index of the shape of the spectrum). We also determined the dissimilarity of composition of zooplankton between each pair of stations using the Bray–Curtis index as a measure of distance between them and then established groups of similar stations by constructing dendrograms of average-linkage, unweighted hierarchical clusters (Legendre and Legendre 1983; Romesburg 1984).

We first calculated Bray–Curtis distances (BCI_1) based on unstandardized (i.e., absolute) values of zooplankton biovolume to estimate dissimilarity between each pair of stations based primarily on the total biovolume of the samples and secondarily on the distributions of the biovolumes into the 12 size categories (eq. 1). In a second set of cluster analyses, we calculated Bray–Curtis distances (BCI_2) for standardized data — zooplankton biovolumes within each size category normalized to the total biovolume in the sample — in order to define dissimilarity between stations based only on the shape of their biovolume spectra (eq. 2). For each pair of stations, the two Bray–Curtis distances are defined as

$$(1)\qquad \mathrm{BCI}_1 = (\Sigma\,|X_{1b} - X_{2b}|)(\Sigma X_{1b} + \Sigma X_{2b})^{-1}$$

$$(2)\qquad \mathrm{BCI}_2 = (\Sigma\,|(X_{1b}(\Sigma X_{1b})^{-1}) - (X_{2b}(\Sigma X_{2b})^{-1})|) \times 0.5$$

where X is biovolume in a particular size category (= bin), 1 and 2 are the respective samples from the two stations, and b is a bin number (1–12) from analysis of each sample.

We generated dendrograms based on each of the two distance measures for the stations of each of the six cruises separately, for all stations in the three cruises of each year, and for those stations from both years where larval hake were found. All dissimilarity matrices and dendrograms were generated with the SPSS Systat statistical package. Stations are successively fused to the station or cluster with the most similar composition (i.e., smallest value of BCI), the resulting clusters becoming progressively fewer, larger, and more heterogeneous until all stations are connected in the dendrogram. We refer to branching points in the dendrogram as "levels."

We tested our hypotheses at three levels in each dendrogram, since there is no test for whether any particular level is statistically significant or not, and since we had no a priori hypotheses as to specifically what property or degree of similarity in the zooplanktonic assemblage might be important to larvae (or might reflect an environment important to larvae). We arbitrarily chose these three levels by compromising between three desiderata: (*i*) at any level within one dendrogram, clusters or groups each composed of at least three stations, and for different dendrograms, (*ii*) approximately the same number of clusters at each level, and (*iii*) approximately the same value of similarity establishing that level. The resulting level at which there are only two clusters, nearest the base of the dendrogram, is designated "primary."

Specifically, we tested null hypothesis H_01b (Table 1) concerning within-year associations between larval hake and zooplankton for each dendrogram at three levels. We tested null hypothesis H_02d for the two assemblages with which larval hake were most strongly associated on the tertiary level of the dendrogram for each year (Table 2).

Table 3. Summary results from cluster analyses.

Comparison	Bray–Curtis equation	Primary level				Secondary level				Tertiary level			
		No. of groups	Statistic	χ^2 or G	p	No. of groups	Statistic	χ^2 or G	p	No. of groups	Statistic	χ^2 or G	p
Within year 1981	1	2	χ^2	15.89	0.00*	6	χ^2	32.49	0.00*	11	G	44.40	0.00*
	2	2	χ^2	1.87	0.17	8	χ^2	9.74	0.20	15	G	28.83	0.01*
Within year 1984	1	2	χ^2	27.72	0.00*	8	G	39.24	0.00*	14	G	42.57	0.00*
	2	2	χ^2	0.28	0.60	5	χ^2	1.75	0.78	9	G	8.27	0.41
1981 and 1984	1	2	χ^2	0.07	0.78	4	χ^2	0.75	0.86	10	χ^2	3.14	0.96
	2	2	χ^2	0.01	0.90	4	χ^2	0.28	0.96	7	G	4.05	0.67

Note: Comparisons within year 1981 or 1984 test whether Pacific hake larvae were distributed nonrandomly with respect to zooplankton clusters for all samples in the January, February, and April cruises of that year ($H_0 1b$, Table 1). Comparisons including both years 1981 and 1984 test whether these years of sampling were distributed nonrandomly among zooplankton clusters, the clusters now consisting only of samples in which larval hake occurred ($H_0 2c$, Table 1). The test statistic G, log likelihood ratio, was used when at least one of the expected frequencies was <1.0 and (or) >20% of the expected frequencies were <5.0 (Zar 1984). The "no. of groups" column indicates the number of zooplankton clusters that resulted from the splitting procedure. An asterisk notes results significant at the α = 0.05 level.

Results

Within-year differences in zooplankton between stations where Pacific hake occurred and those where they did not

Because larval hake were rare in May (Figs. 2 and 3), the preliminary search for differences between those stations where larvae occurred and those where larvae were absent (i.e., $H_0 1a$) was confined to January, February, and April. In 1981, the median total biovolume of zooplankton, the median biovolume of small (750–860 µm ESD) zooplankton, and the median biovolume ratio where larvae were encountered were significantly greater than the comparable medians for stations without larvae (140 and 178 stations, $p < 0.01$, two-tailed, in all cases, see Table 2). The same differences were significant in 1984 (87 and 265 stations, $p < 0.01$, two-tailed, in all cases, see Table 2). Within each year, the total biovolume and small zooplankton biovolume were negatively correlated with temperature at 10 m (a surrogate for the physical environment), but the biovolume ratio was not ($H_0 1Aa$ accepted).

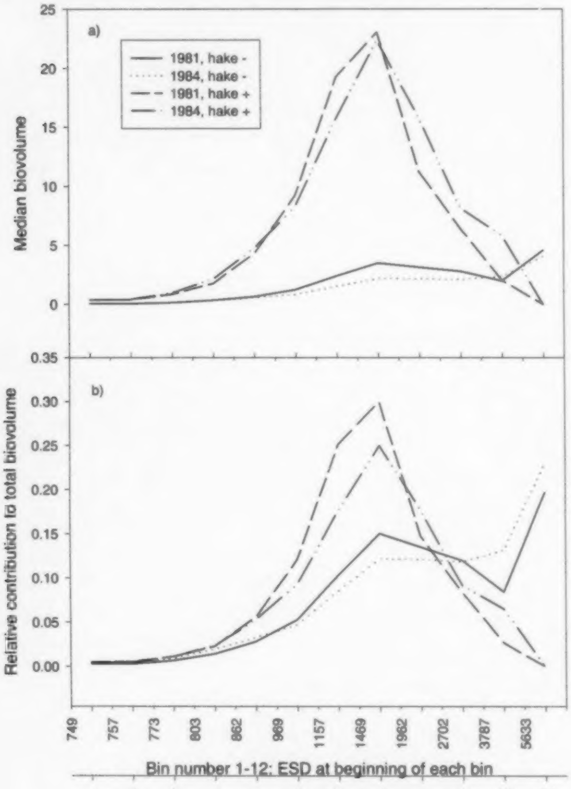
Larval hake occurred nonrandomly with respect to macrozooplanktonic assemblages identified by the 1981 and 1984 dendrograms (January, February, and April cruises in each year) based on the unstandardized data matrix (BCI_1, eq. 1) at all three levels of clustering (Table 3). Analyses of five cruises separately yielded similar results for at least one of the levels (e.g., in 1984, the larvae in February, and in April, each had significant nonrandom associations with zooplankton at both the secondary and tertiary levels). The January 1984 cruise was not analyzed due to the sparcity of stations at which larval hake were found.

We ranked clusters from the 1981 and 1984 dendrograms at the tertiary level according to the strength of larval hake's association with them and then plotted the median biovolume in each size category for the assemblages with which larvae were most and least associated (Fig. 4*a*). This comparison suggests that the total biovolume of zooplankton and the relative upward convexity of the biovolume spectrum, reflecting large relative biovolume in categories 7–9 (1.16–2.70 mm ESD), were two features leading to strong association by larval hake. This finding led us to establish the simple "biovolume ratio" described in the Materials and methods and the rank sum tests whose outcomes are shown in Table 2.

The occurrence of larval hake with zooplanktonic assemblages derived from data standardized to the total biovolume (BCI_2, eq. 2) was significant in only one case, the tertiary level of the 1981 dendrogram (Table 3). A plot of median values of standardized biovolume per size category for the tertiary level of the 1981 dendrogram supported our conclusion that a concentration of biovolume in categories 7–9 (1.16–2.70 mm ESD) was an important characteristic of assemblages with which larval hake are associated (Fig. 4*b*)

In summary, $H_0 1a$ and $H_0 1b$ (Table 1) generally were rejected for analyses where absolute biovolume was an important factor in characterizing zooplanktonic assemblages. Where spectral shape was the only factor — the biovolume ratio and the dendrograms based on standardized biovolumes — the former led to convincing rejection of $H_0 1a$

Fig. 4. Median biovolume spectrum for the zooplankton cluster in each year's tertiary-level dendrogram analysis with which larval Pacific hake were most associated in that year and the cluster with which larvae were least associated. The abscissa shows bin numbers 1–12 and the ESDs (μm) separating the bins. The 1981 and 1984 "hake+" lines are median spectra from 33 and 29 samples, respectively (comprising the clusters with which larval hake were most positively associated). The 1981 and 1984 "hake–" lines are median spectra from 55 and 23 samples, respectively (the clusters with which the association of larval hake was most negative). (*a*) absolute biovolumes, (*b*) relative biovolumes.



(and, importantly, also acceptance of $H_0 1Aa$), but rejection of $H_0 1b$ was at best weak.

Differences in zooplankton between 1981 and 1984

Based on all four cruises in each year, the median total biovolume, median biovolume of small zooplankton, and median biovolume ratio were all greater in 1981 than in 1984 ($H_0 2a$, $p < 0.01$ by rank sum test in all cases, see Table 2). These results are inconsistent with the hypotheses that either total or small zooplankton biovolume is an accurate surrogate for larval food and that successful recruitment is food limited, since both biovolumes were less in the year of remarkably successful recruitment. The results are also inconsistent with the alternative hypothesis that a large biovolume ratio in the zooplankton indicates a year of strong recruitment of hake.

Table 4. Nearshore occurrence of Pacific hake larvae in winter–spring 1981 and 1984 ($H_0 3a$, Table 1).

	Fraction nearshore (%)	
Month	1981	1984
January	49	70
February	14	42
April	28	27
May	37	12
Overall	20	41
Overall without dominant station	39	41

Note: Tabulated is the fraction of larvae within each cruise and year occurring in the nearshore zone depicted in Figs. 2 and 3. Larval abundances are weighted by the area represented by each station, and abundances at stations along the boundary are split between nearshore and offshore zones.

Because it is possible that there were important differences between years in those stations where hake occurred most regularly — differences that were obscured when the entire suite of stations was considered — we examined the subsets consisting of those stations where larval hake occurred in each year and the 47 stations at which larvae occurred in the same month in both years (cf. $H_0 2b$, Table 1). In neither case were there significant differences between years in either the total biovolume of zooplankton or biovolume of the small (750–860 μm ESD) zooplankton (which is closer to the sizes of zooplankters that larval hake actually eat; see above). As was true for all stations, the median biovolume ratio at stations where larvae occurred in 1981 was greater than that in 1984 (Table 2), but the acceptable alternative hypothesis again is not consistent with the finding that, within years, a large biovolume ratio indicated a station that was "good" for larvae.

Thus, although within-year comparisons indicated that larval hake were associated with relatively large biovolumes, large biovolumes of small zooplankton, and a large biovolume ratio, these factors did not explain why recruitment in 1984 was so much better than in 1981. Hypothesis $H_0 2b$ could not be rejected in favor of an alternative hypothesis that strong years in recruitment could be explained by the same factors that, within years, distinguished stations where larvae occurred from those where they did not.

Cluster analyses of unstandardized (BCI_1, eq. 1) or standardized (BCI_2, eq. 2) data combining the two years, including only those stations where larval hake were present, yielded dendrograms in which there was random assortment of the samples from each year into the clusters (Table 3). That is, null hypothesis $H_0 2c$ (Table 1) was not rejected. This further suggests that differences between the two years in zooplankton biovolume or spectra were not related to the differences in recruitment.

Similarly, a rank sum test comparing the biovolume ratio (upward convexity of the spectrum) of samples comprising the two clusters with which larval hake were most strongly associated in 1981 and in 1984 (three cruises per year, clusters from tertiary level of dendrograms of BCI_1, eq. 1) was not significant (Table 2). Thus, $H_0 2d$ was not rejected; the relative size distribution of biovolume at that cluster of stations with which larval hake were most associated did not

Fig. 5. Hydrographic sections for four cruises in 1984 along the nearshore/offshore boundary shown in Fig. 3, starting at line 60 in the north. Isopleths of density (σ_t) are shown, together with the geostrophic transport in the 50- to 150-m layer (shown on the broken line at 100 m) relative to 500 m. Symbols in the cruise 8405 panel indicate directions (×, offshore; ○ onshore) and speeds of geostrophic currents.

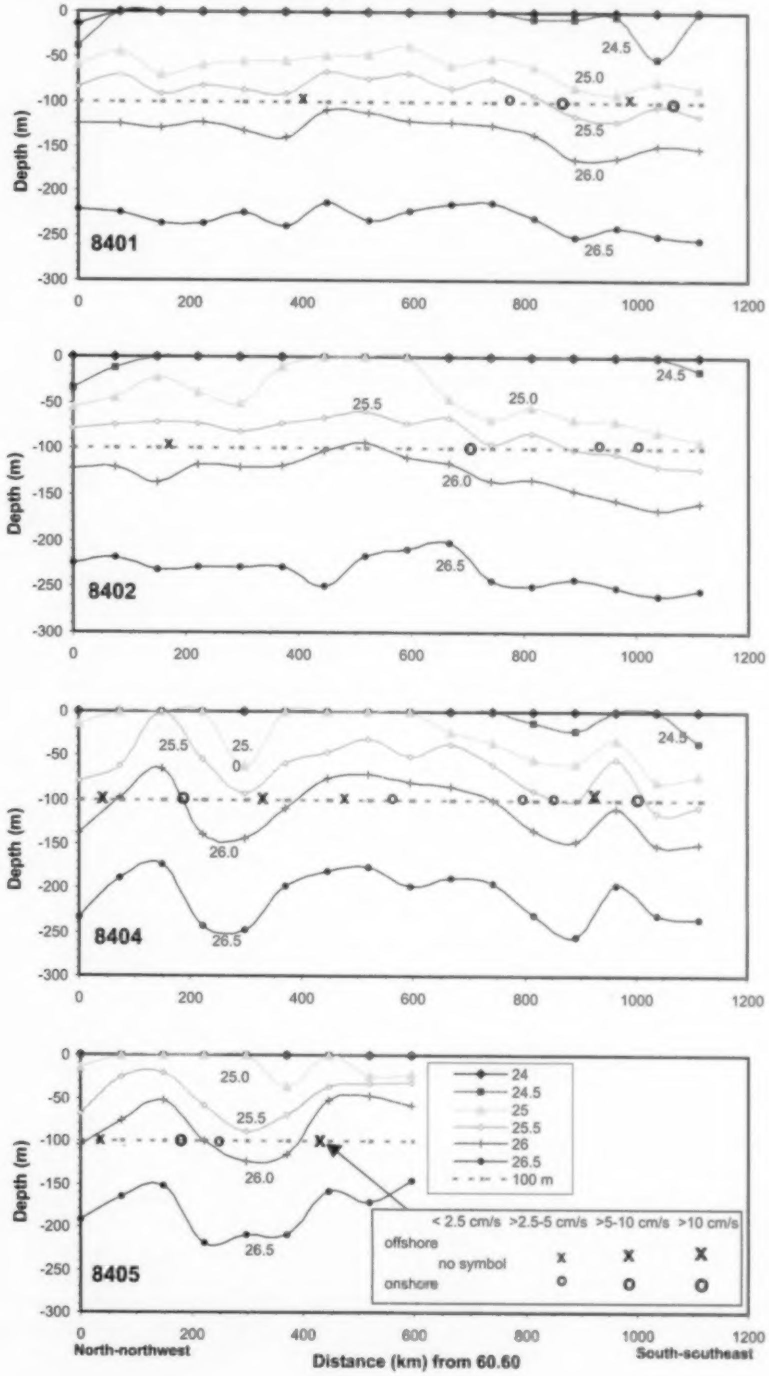


Table 5. Proxies for larval survival in 1981 and 1984, the higher number in each column indicating the year of greater survival ($H_0$4, Table 1).

	Jan.Feb.+Apr.				
Year	>3.5/≤3.5	>4/≤4	>4.5/≤4.5	>5/≤5	Cohort following, Jan.+Feb. to Apr., >10/≤3.5
1981	0.53	0.42	0.36	0.20	0.06
1984	0.48	0.26	0.16	0.10	0.04

Note: The proxies were ratios of abundances of larger (presumably older) to smaller (presumably younger) larvae, calculated for separations made at four different lengths (mm). The resulting four ratios of larger to smaller larvae for "Jan.Feb.+Apr." represent all larvae captured in these months; the final ratio ("cohort following, Jan.+Feb. to Apr.") is the abundance at all stations of larvae >10 mm long in April relative to the abundance of larvae ≤ 3.5 mm in January + February, which approximates the proxy used by Bailey (1981).

differ between years (p = 0.066). We conclude that differences between the years in success of recruitment could not be explained by differences in the characteristics of the zooplanktonic groups with which larval hake were associated.

Nearshore/offshore occurrence and transport

In 1981 (the year-class with poor recruitment), only 20% of the larvae in the months surveyed occurred nearshore (Table 4). This outcome must be interpreted cautiously, however, as it is strongly affected by a very large catch at a single station just offshore of the boundary in February, where 49% of all the larvae caught in 1981 were found (Fig. 2*b*). If this one station is removed from the calculation, 39% of the larvae in 1981 were nearshore (Table 4), a result that is quite similar to that for 1984. A single station sampled in February also dominated the results for 1984, accounting for 66% of the larvae caught that year. Unlike the dominant station in 1981, this station was situated on the nearshore/offshore boundary (Fig. 3*b*), so half of its larvae were "nearshore." Because its catch was split, removing this station from consideration does not change the overall result for 1984 (Table 4).

The overall fraction of larvae that were found in the nearshore zone in 1984 was therefore greater than in 1981, but this difference is due largely to the slight difference in location, relative to the arbitrary boundary, of the stations dominating each year's catch of larvae. Although it is possible that unusually successful recruitment of larvae from a single location of mass spawning could establish a strong year-class, there is little evidence that the unusual success of 1984 year-class was due to such site-specific recruitment. Given the arbitrariness of the nearshore/offshore boundary, and the patchiness of sampling, differences between dominant stations in 1981 and 1984 are slight. Nor do analyses of geostrophic transport or upwelling (below) reveal a mechanism by which 1984's dominant station for larvae, but not 1981's most important station, would dominate the subsequent recruitment also.

In 1984, there was little overall trend in geostrophic transport onshore versus offshore (Fig. 5). In February, when larvae were widespread and numerous (Fig. 3), the stations where abundant larvae were found were primarily in the north, while advection onshore was primarily in the south (Fig. 5, section for cruise 8402). Further, the single station at which 66% of the larvae for the year occurred (Fig. 3*b*) was at 220 km on this section; just to the north, geostrophic flow was offshore. Although geostrophy is not an ideal indicator of cross-shelf flow, if this section is representative of longer-term advection of larvae, and if "nearshore" = "good," there is little evidence that larvae were being advected shoreward so as to cause improvement in eventual recruitment.

Evaluating all the (admittedly imperfect) evidence, it is difficult to argue that the success of recruitment in 1984 relative to 1981 was due to more felicitous placement of larvae in the nearshore/offshore sense. Qualitatively, $H_0$3*a* (Table 1) must be accepted.

The winters and springs of both 1981 and 1984 were characterized by upwelling-favorable winds. Upwelling (as calculated by the Pacific Fisheries Environmental Laboratory) was greater in January–May of 1984 than in 1981 except in April at the northernmost location (39°N, 125°W) and in May at all but the southernmost location (30°N, 119°W). When weighted for larval abundances in time and space (see Materials and methods), significantly more "larval-relevant" upwelling occurred in 1984 than in 1981 ($H_0$3*b* rejected, $p <$ 0.025 by two-tailed signed rank test). There were upwelling-favorable winds around 36°N, 122°W in February 1984, very near the station at which the majority of larvae were found (Fig. 3*b*).

Because upwelling changes seasonally, we also examined the anomalies from long-term monthly means. Out of 20 possible location/month anomalies, all but four were positive in winter–spring 1984. Hence, upwelling in 1984 was generally stronger than the norm. Upwelling near the most important station for larvae in 1984 (Fig. 3*b*) in February at 36°N, 122°W, although positive, was slightly less than the long-term monthly mean (i.e., a negative anomaly). However, the negative anomaly in upwelling near the most important station for larvae in 1981 (February at 33°N, 119°W) (Fig. 2*b*) was even greater. Hence, there is little evidence that interannual differences in either overall upwelling or the upwelling at the most important station in each year were correlated with strong recruitment in 1984.

Larval survival between years

We examined several ratios of abundances of larger (older) to smaller (younger) larvae, using several sizes as proxies for age, in order to minimize the effects of vagaries in sampling and measurement. Table 5 lends no support to the hypothesis that the successful recruitment of 1984 was due to unusually high larval survival. All the proxies for survival were less in 1984 than in 1981, a result that, if it indicates rejection of $H_0$4 (Table 1), does so in the opposite sense than the anticipated alternative.

Discussion

Interannual variation in recruitment

Recruitment of the 1984 year-class of Pacific hake was unusually strong, but our comparison of 1984 and 1981 did not support any of several possible causal mechanisms acting during the larval phases proposed in previous studies (based primarily on data from earlier years) or our own intuition that the size spectrum of zooplankton might be a significant variable. Relative to 1981, biomass of small zooplankton (a proxy, although a poor one, for availability of food) and the convexity of the size spectrum in 1984 were less (although not always significantly so). Recall that the convexity of the size spectrum of zooplankton is not a proxy for food; it is a purely empirical index of some property (other than temperature) that is related to zooplankton and with which larval hake tend to be nonrandomly associated *within* each year.

Larval survival tended to be less in 1984 than in 1981. Nearshore occurrence of larvae was not remarkable in 1984 (if one particular station in 1981 was removed from the comparison). Bailey and Francis (1985) argued that strong year-classes tended to occur in years of downwelling or weak upwelling. Our analysis indicates that the 1984 year-class did not fit this pattern, since upwelling over the whole area in winter–spring was both stronger than in 1981 and strong relative to the long-term monthly means. Upwelling was anomalously weak in the vicinity of the most important single station in 1984, but even more so for the most important single station in 1981.

Our results do not necessarily vitiate earlier findings because we compared only two years, used surrogates rather than direct measurements of several factors, and analyzed the surrogates differently than did previous authors. However, our results clearly do not support any of the proposed causal mechanisms (within the larval phase) for good recruitment. One interpretation is that recruitment success actually is determined (or was determined in 1984) in early juvenile rather than larval life, a possibility that we were unable to examine.

Validation by predicting where larval hake occurred in spring 1998

In the winter–spring period of 1981 and 1984, larval hake tended to occur at stations characterized by a particular size distribution of macrozooplankton. This association was not due simply to parallel, but independent, correlations of larvae and the size distribution with temperature. Even without exact understanding of the causation behind it, this association could have predictive capacity and might be useful as a way of finding stations where larval hake would likely be found.

In 1998, the CalCOFI program again sampled a region large enough to include much of the spawning area of hake, although sampling did not extend into Mexican waters. We were therefore able to test whether larval hake again were associated with a particular size spectrum of zooplankton, using the preserved samples. Further, because measurements also were made with an in situ, internally recording OPC at many stations where net tows were made, it was possible to restrict the examination of the size spectra of zooplankton to depths below 50 m, where larvae typically occur (cf. Mullin and Cass-Calay 1997). We did this even though changes in zooplankters due to preservation affect their detection by an OPC (Beaulieu et al. 1999), and there are other differences between the laboratory and in situ measurements (e.g., avoidance of the in situ OPC by living zooplankters).

Analysis of 59 preserved samples showed that, as in 1981 and 1984, larval hake were found disproportionately at those stations where (1) macrozooplanktonic biomass was large (median for stations where larvae occurred = 70 $\mathrm{mm^3/m^3}$ and where larvae were absent = 34 $\mathrm{mm^3/m^3}$; significantly different by rank sum test, $p < 0.01$) and (2) where the biovolume ratio (see Materials and methods) was large (median for stations where larvae were present = 2.0 and where larvae were absent = 1.3; significantly different by rank sum test, $p < 0.01$) (cf. Table 2).

In this sense, the findings from the early 1980s proved to be robust. However, unlike the lack of correlation between biovolume ratios and temperatures over the four winter–spring cruises in 1981 and over the four in 1984 (hypothesis $H_0 1Aa$ (Table 1) accepted), for this single cruise in 1998, there was a significant, negative correlation between biovolume ratios and temperatures at the same stations.

In situ OPC data, taken at many (but not all) of these same stations in spring 1998, demonstrated that the biovolume ratio of zooplankton in the 50- to 150-m layer (approximately the vertical distribution of larval hake) was positively correlated with the biovolume ratio of zooplankton determined by this instrument from the surface to the bottom of the tow (equivalent to the column integrated by net sampling and analyzed in the laboratory, $p < 0.025$, one-tailed). In spite of this, and in contrast with conclusion 2 above (analysis of 0- to 200-m preserved samples), the median biovolume ratio determined in the 50- to 150-m layer by the in situ OPC at stations where larval hake were found did not differ significantly from the median at stations without hake.

This discrepancy in outcomes could simply be due to analyzing fewer stations with the in situ OPC than were analyzed from preserved zooplankton samples. We therefore reanalyzed the data taken in the laboratory using the preserved samples, but for only those stations at which in situ OPC data had also been taken. The biovolume ratios estimated by the in situ and laboratory techniques were positively correlated ($p < 0.025$, one-tailed, for $N = 48$). For those stations where in situ data were taken, the median biovolume ratio of the preserved zooplankton where larval hake were encountered was significantly greater than that where hake were absent, as for the larger number of stations in spring 1998, where only preserved samples were analyzed.

Thus, the discrepancy in conclusions between analyses of preserved zooplankton and in situ analyses in spring 1998 cannot be attributed to analyzing data from 50 to 150 m only or to the limited set of stations at which in situ data were taken. Unless discrepancy in conclusions is a statistical artifact, it appears that the difference was somehow due to differences between how the in situ OPC counts and measures living zooplankton and how the laboratory OPC counts and measures preserved ones. Until these differences are resolved, one could not use the biovolume ratio determined by the in situ OPC as a way of searching for stations where larval hake are likely to occur.

Acknowledgments

Students Eric Nelson, Elizabeth Taylor, Grace Tsai, Solana Pyne, and Michael Thimgan assisted in analyzing samples during this project. Martin Dorn kindly provided data on recruitment of hake, and Richard Charter helped us find and recover relevant data concerning the cruises. David Checkley provided the OPC and designed the equipment for diluting samples prior to flow to the OPC, which was constructed by Van Tang Thieu. Annie Townsend provided us with samples from the Scripps Institution of Oceanography Pelagic Invertebrates Collection. Kirk Ireson assisted with the in situ OPC study in 1998. The Pacific Fisheries Environmental Laboratory, NMFS, NOAA, provided monthly means of coastal upwelling, computed from the atmospheric pressure fields. Financial support was provided by a grant from the National Sea Grant College Program, NOAA, U.S. Department of Commerce, under grant No. NA66RG0477, project No. R/CZ-13B, through the California Sea Grant College System. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. The U.S. Government is authorized to reproduce and distribute for governmental purposes.

References

Ahlstrom, E.H. 1959. Vertical distribution of pelagic fish eggs and larvae off California and Baja California. Fish. Bull. U.S. **60**: 107–146.

Alheit, J., and Pitcher, T.J. (*Editors*). 1995. Hake: biology, fisheries and markets. Chapman and Hall, London, U.K.

Ambrose, D.A., Charter, R.L., Moser, H.G., and Earhart, B.S. 1988. Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1981. NOAA-TM-NMFS-SWFC-112. U.S. Department of Commerce, Washinton, D.C.

Bailey, K.M. 1980. Recent changes in the distribution of hake larvae: causes and consequences. Calif. Coop. Oceanic Fish. Invest. Rep. **21**: 167–171.

Bailey, K.M. 1981. Larval transport and recruitment of Pacific hake *Merluccius productus*. Mar. Ecol. Prog. Ser. **6**: 1–9.

Bailey, K.M., and Francis, R.C. 1985. Recruitment of Pacific whiting, *Merluccius productus*, and the ocean environment. Mar. Fish. Rev. **47**: 8–15.

Bailey, K.M., and Yen, J. 1983. Predation by a carnivorous marine copepod, *Euchaeta elongata* Esterly, on eggs and larvae of the Pacific hake, *Merluccius productus*. J. Plankton Res. **5**: 71–82.

Bailey, K.M., Francis, R.C., and Stevens, P.R. 1982. The life history and fishery of the Pacific whiting, *Merluccius productus*. Calif. Coop. Oceanic Fish. Invest. Rep. **23**: 81–98.

Beaulieu, S.E., Mullin, M.M., Tang, V.T., Pyne, S.M., King, A.L., and Twining, B.S. 1999. Using an optical plankton counter to determine the size distributions of preserved zooplankton samples. J. Plankton Res. **21**: 1939–1956.

Butler, J.L., and Nishimoto, R.N. 1997. Growth and cohort dynamics of larval Pacific hake (*Merluccius productus*). Calif. Coop. Oceanic Fish. Invest. Rep. **38**: 63–68.

Cass-Calay, S.L. 1997. Relation of mean growth rate to concentration of prey-sized particles for larvae of Pacific hake, *Merluccius productus*. Calif. Coop. Oceanic Fish. Invest. Rep. **38**: 69–76.

Chelton, D.B., Bernal, P.A., and McGowan, J.A. 1982. Large-scale interannual physical and biological interaction in the California Current. J. Mar. Res. **40**: 1095–1125.

Dorn, M.W. 1996. Status of the coastal Pacific whiting resource in 1996. *In* Pacific Fishery Management Council. Status of the Pacific Coast groundfish fishery through 1996 and recommended acceptable biological catches in 1997. Pacific Fishery Management Council, Portland, Oreg. pp. A1–A77.

Francis, R.C. 1983. Population and trophic dynamics of Pacific hake (*Merluccius productus*). Can. J. Fish. Aquat. Sci. **40**: 1925–1943.

Herman, A.W. 1988. Simultaneous measurement of zooplankton and light attenuance with a new optical plankton counter. Continental Shelf Res. **8**: 205–221.

Herman, A.W. 1992. Design and calibration of a new optical plankton counter capable of sizing small zooplankton. Deep-Sea Res. **39**: 395–415.

Hollowed, A.B. 1992. Spatial and temporal distributions of Pacific hake, *Merluccius productus*, larvae and estimates of survival during early life stages. Calif. Coop. Oceanic Fish. Invest. Rep. **33**: 100–123.

Hollowed, A.B., Hare, S.R., and Wooster, W.S. 1998. Pacific basin climate variability and patterns of northeast Pacific marine fish production. *In* Proceedings of the 10th 'Aha Huliko,'a Hawaiian Winter Workshop, Biotic Impacts of Extratropical Climate Variability in the Pacific. *Edited by* G. Holloway, P. Muller, and D. Henderson. Spec. Publ., School of Ocean and Earth Science and Technology University of Hawaii at Manoa. pp. 89–104.

Horne, J.K., and Smith, P.E. 1997. Space and time scales in Pacific hake recruitment processes: Latitudinal variation over annual cycles. Calif. Coop. Oceanic Fish. Invest. Rep. **38**: 90–102.

Legendre, L., and Legendre, P. 1983. Numerical ecology. Elsevier Science Publishers, Amsterdam, The Netherlands.

Moser, H.G., Charter, R.L., Smith, P.E., Ambrose, D.A., Charter, S.R., Meyer, C.A., Sandknop, E.M., and Watson, W. 1993. Distributional atlas of fish larvae and eggs in the California Current region: taxa with 1000 or more total larvae, 1951 through 1984. Calif. Coop. Oceanic Fish. Invest. Atlas No. 31.

Mullin, M.M., and Cass-Calay, S.L. 1997. Vertical distributions of zooplankton and larvae of the Pacific hake (whiting), *Merluccius productus*, in the California Current System. Calif. Coop. Oceanic Fish. Invest. Rep. **38**: 127–136.

Roemmich, D. 1992. Ocean warming and sea level rise along the southwest U.S. coast. Science (Washington, D.C.), **257**: 373–375.

Romesburg, H.S. 1984. Cluster analysis for researchers. Lifetime Learning Publ., Belmont, Calif.

Scripps Institution of Oceanography. 1984*a*. Physical, chemical and biological data, CalCOFI cruise 8401, 4–27 January 1984. SIO Ref. 84-18. Scripps Institution of Oceanography, University of California San Diego, La Jolla, Calif.

Scripps Institution of Oceanography. 1984*b*. Physical, chemical and biological data, CalCOFI cruise 8402–3, 7 February – 30 March 1984. SIO Ref. 84-23. Scripps Institution of Oceanography, University of California San Diego, La Jolla, Calif.

Scripps Institution of Oceanography. 1984*c*. Physical, chemical and biological data, CalCOFI cruise 8404, 9 April – 4 May 1984; CalCOFI cruise 8405, 14 May – 2 June 1984. SIO Ref. 84-25. Scripps Institution of Oceanography, University of California San Diego, La Jolla, Calif.

Stevens, E.G., Charter, R.L., Moser, H.G., and Meyer, C.A. 1990. Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1984. NOAA-

TM-NMFS-SWFC-141. U.S. Department of Commerce, Washington, D.C.

Sumida, B.Y., and Moser, H.G. 1980. Food and feeding of Pacific hake larvae, *Merluccius productus*, off Southern California and northern Baja California. Calif. Coop. Oceanic Fish. Invest. Rep. **21**: 161–166.

Zar, J.H. 1984. Biostatistical analysis. Prentice Hall, Englewood Cliffs, N.J.

Geographic variation in the sex ratio, sex specific size, and age structure of *Plectropomus leopardus* (Serranidae) between reefs open and closed to fishing on the Great Barrier Reef

S. Adams, B.D. Mapstone, G.R. Russ, and C.R. Davies

Abstract: Variation in reproductive parameters of *Plectropomus leopardus* populations between coral reefs open and closed to fishing was examined in two geographic regions (off Townsville (19°S) and in the Swains (22°S)) of the Great Barrier Reef, Australia. The reproductive parameters that were examined included the operational (reproductive) sex ratio and size and age structure of mature females, sex-changing individuals (transitionals), and mature males. There was a greater proportion of males above the minimum size and age of harvest on reefs closed to fishing for 8–10 years than on reefs open to fishing, although the effect varied with geographic region. Nevertheless, neither the sex ratio, mean age, nor mean size of males or transitionals varied significantly with reef closure status. Females, however, were significantly older and larger on closed than on open reefs in both regions. Marked regional variations in the sex ratio and average age of transitionals were found independent of reef closure status, suggesting regional variation in reproductive strategies. Controlled manipulations, inclusion of broad spatial scales, and detailed studies of intra- and inter-specific variation in serranid reproductive biology are necessary to resolve such complexities in the responses of protogynous serranids to fishing pressure and management strategies.

Résumé : Nous avons examiné la variation des paramètres de reproduction des populations de *Plectropomus leopardus* dans des récifs coralliens, certains ouverts, d'autres fermés à la pêche, dans deux régions géographiques (au large de Townsville, 19°S et dans les Swains, 22°S) du récif de la Grande Barrière, en Australie. Les paramètres de reproduction examinés étaient le rapport des sexes opérationnel (reproduction), la taille et la structure par âge des femelles matures, des individus en transition (changement de sexe) et des mâles matures. On notait sur les récifs fermés à la pêche depuis 8 à 10 ans une plus forte proportion de mâles au-dessus de la taille et de l'âge minimums de capture que sur les récifs ouverts à la pêche, mais l'effet variaient selon la région. Toutefois, ni le rapport des sexes, ni l'âge moyen ou la taille moyenne des mâles ou des individus en transition ne variait de façon significative en fonction de l'ouverture ou de la fermeture de la pêche. Les femelles étaient toutefois, dans les deux régions, nettement plus âgées et plus grosses dans les zones fermées que dans les zones ouvertes. Les variations régionales marquées du rapport des sexes et de l'âge moyen des individus en transition étaient indépendantes de l'ouverture ou de la fermeture de la zone, ce qui évoque l'existence d'une variation régionale dans les stratégies de reproduction. Il serait nécessaire d'avoir recours à des manipulations contrôlées, d'inclure de grandes échelles spatiales et de procéder à des études détaillées sur la variation intra- et inter-spécifique dans la biologie de reproduction des serranidés pour élucider les problèmes complexes que pose la réaction des serranidés protogynes à la pression de pêche et aux stratégies de gestion.

[Traduit par la Rédaction]

Introduction

Epinepheline serranids (groupers, coral cods) are highly valuable food fish in the tropics (Heemstra and Randall 1993). Most are also protogynous hermaphrodites (change sex from female to male). Management of fishery species may be complicated by protogynous sexual development, as sex change is often not accounted for in traditional management models (Bannerot et al. 1987). It has been suggested that species with protogynous sexual strategies under these conditions are more vulnerable to fishing pressure than targeted species that have separate sexes throughout life (Punt et al. 1993; Huntsman and Schaaf 1994).

Fishing is considered to have potentially detrimental consequences on the reproductive capacity of protogynous populations by causing sperm limitation via the disproportionate removal of larger, presumably male individuals (uncompensated response; Coleman et al. 1996). Such effects are expected where sex change occurs at a fixed size or age and (or) the rate of male removal exceeds the rate that males can be replaced in the population (Vincent and Sadovy 1998). However, this is largely based on generalized assumptions of

Received June 25, 1999. Accepted March 29, 2000.
J15219

S. Adams[1] and G.R. Russ. Department of Marine Biology, James Cook University, Townsville, Queensland, Australia 4811.
B.D. Mapstone and C.R. Davies. CRC Reef Research Center, Department of Tropical Environmental Studies and Geography, James Cook University, Townsville, Queensland, Australia 4811.

[1] Author to whom all correspondence should be addressed. e-mail: samantha.adams@jcu.edu.au

the reproductive biology of the group and the perceived targeting behavior of fishers. Recent evidence suggests that epinepheline serranids can show substantial variation in reproductive patterns (Sadovy and Colin 1995) and, consequently, are likely to show differing responses to fishing pressure. Furthermore, most studies examining the effect of fishing on the sex ratio and (or) the sex-specific size and age structure of protogynous serranids have not replicated fished and unfished areas (e.g., Thompson and Munro 1983). This is largely due to the difficulty in locating unfished sites. Consequently, what may appear to be a typical uncompensated response to fishing cannot be separated conclusively from natural variation in one or more reproductive parameters.

In general, fisheries selectively remove larger, older individuals from the population (e.g., Jennings and Lock 1996). In protogynous species, the larger individuals are most likely to be male, and their disproportionate removal can be expected to affect a number of reproductive parameters of the population (Huntsman and Schaaf 1994). Hence, changes in the sex ratio and (or) the size and age structure of male and transitional individuals have been considered the best reproductive parameters to indicate an effect of fishing. The sex ratio is likely to be insensitive to effects of fishing if sex change is socially mediated and the females of the population compensate for male removal by changing sex earlier (compensating response; Shapiro 1981, 1987). In this case, a reduction in the mean size and age of females, transitionals, and males is likely to occur following the removal of the larger males (Vincent and Sadovy 1998). Although the sex ratio of the population is maintained, this response could lead to a reduction in population fecundity due to the decreased average female size (Sadovy 1996).

The protogynous, epinepheline serranid *Plectropomus leopardus* (common coral trout) is the major target species of the hook-and-line fishery on Australia's Great Barrier Reef. The rapid development and high market value of *P. leopardus*, particularly in the context of the recent trade in live reef fish from Australia to Asia, have created public concern about the status of *P. leopardus* stocks along the Great Barrier Reef (Mapstone et al. 1996*a*). The two regions examined in this study have the highest records of historical fishing effort across the entire Great Barrier Reef, averaging approximately 4000 main boat-days per year, with a maximum of 9000 boat-days per year (Mapstone et al. 1996*a*).

The Great Barrier Reef is characterized by over 2700 spatially segregated platform reefs and shoals, many of which have been zoned Marine National Park (fishing prohibited) for several years. Mapstone et al. (1998) estimated that fishing effort was significantly greater around reefs open to fishing (open reefs) than around reefs zoned Marine National Park (closed reefs). Further, mortality estimates of *P. leopardus* have been found to be approximately three times higher on open reefs than on closed reefs, suggesting that fishing can substantially affect populations on unprotected reefs (Russ et al. 1998). After postlarval settlement onto reefs, adult *P. leopardus* show minimal movement between reefs (Davies 1995), even during spawning periods (Zeller 1998; Zeller and Russ 1998). The low flux rates of adult *P. leopardus* between reefs, coupled with the spatial arrangement of open and closed reefs, provided the opportunity for a retrospective comparison of the operational sex ratio and sex-specific size and age structure between *P. leopardus* populations that had been protected and unprotected from fishing. The potential effects of fishing on these reproductive parameters can then be implied (Mapstone et al. 1996*b*).

Successful closure of reefs to fishing would be expected to result in a larger and older spawning stock of *P. leopardus* relative to open reefs (Mapstone et al. 1996*b*). The extent of female bias in the operational sex ratio that causes sperm limitation in noncompensating protogynous populations is unknown. However, according to conventional theory, populations on reefs open to fishing would be expected to have a significantly higher female bias than those on closed reefs (Bannerot et al. 1987). Ferreira and Russ (1995) and Russ et al. (1995) made such an assessment after an average of 4 years of protection from fishing in one geographic region of the Great Barrier Reef only. The study that we report here was done in two regions separated by over 3° of latitude and included eight reefs (two open and two closed to fishing within each geographical region) with periods of reef closures of 8–10 years. The spatial scale, replication of reefs, and period of reef closure in this study make it the most extensive to date. We compared the operational sex ratio and size and age structure of mature female, transitional, and mature male *P. leopardus* between open and closed reefs in the two geographic regions in order to examine both large-scale variation in reproductive biology and whether larger, older spawning stocks and (or) a significantly less female-biased sex ratio were associated with closed reefs relative to open (harvested) reefs.

Materials and methods

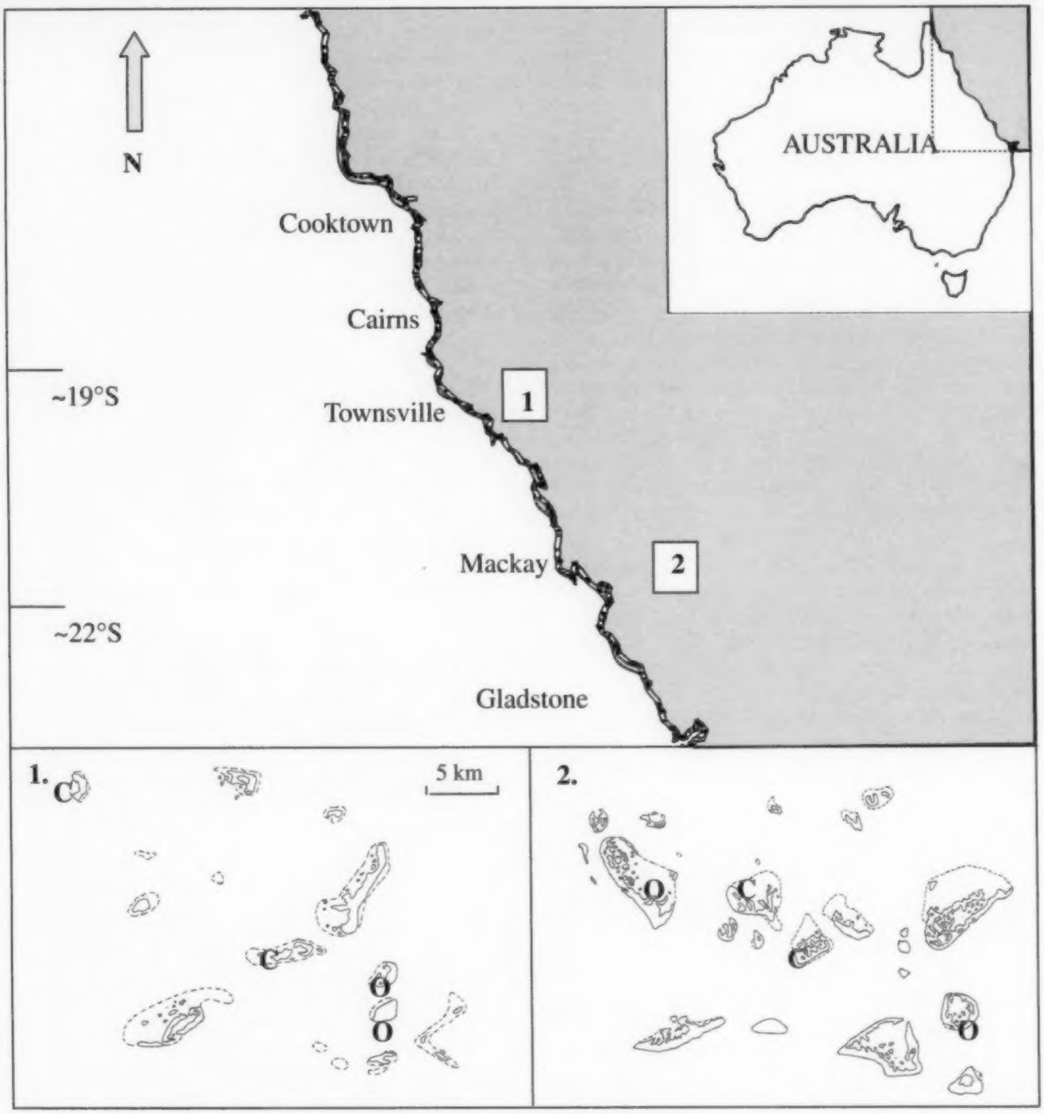
Field sampling

We examined the sexual structure of *P. leopardus* sampled from clusters of reefs in two regions, one off Townsville (the Townsville cluster (19°S)) and one in the Swains (the Swains cluster (22°S)) (Fig. 1). Samples were obtained from research line fishing surveys conducted as part of the monitoring program associated with the "effects of line fishing experiment" (Davies et al. 1998; Mapstone et al. 1998). Logistic constraints prevented concurrent sampling in both regions. Reefs in the Townsville region were sampled early in the spawning season and reefs in the Swains region late in the spawning season in 1995. *Plectropomus leopardus* aggregate to spawn on each reef around the new moons in the austral spring to early summer but disperse (within reefs) from aggregations between new moons (Samoilys 1997). Both regions were sampled in the week of a full moon within the spawning season, as this is the "off-peak" period of spawning activity when individuals are ripe but nonaggregated (Samoilys 1997). This sampling strategy minimized potential for confounding effects of variations in aggregating behavior with regional effects.

Approximately 200–300 *P. leopardus* per reef were caught by professional fishers using standardized hook-and-line gear under research supervision. Four reefs were examined within each cluster: two reefs that had been zoned as Marine National Park B (closed to fishing) for more than 8 years and two zoned General Use (open to line fishing). For brevity, these management zones will be referred to collectively as "reef closure status" and individually as "closed" and "open" (to fishing) reefs, respectively.

Sampling around each reef was highly structured. The perimeter of each reef was divided into six equal-sized "blocks" and fishing effort was distributed equally among blocks and across two depth

Fig. 1. Location of sampled reefs open (O) and closed (C) to fishing off Townsville (1) and in the Swains region (2), Great Barrier Reef, Australia.



ranges (shallow, <12 m; deep, 12–45 m) within each block. Fishers and gear were standardized throughout the survey. Methodologically consistent samples were therefore obtained for all reefs, above 45 m depth, which provided for logically sound comparisons among all reefs. Size selectivity of the gear reaches 50% at approximately 31.5 cm fork length (FL) and 100% at 38 cm FL (Fulton 1996). The size at 100% maturity for female *P. leopardus* was found to be approximately 35 cm FL (Adams 1996), and thus, the majority of the mature population was available to the sampling gear. Fish were killed on capture, individually tagged, measured to the nearest millimetre FL, placed in a seawater ice slurry, and processed at sea (gilled, gutted, and (or) filleted) at the end of each fishing day. Frames of caught fish were frozen following processing. Upon return to the laboratory, gonads were removed and weighed, when possible, sex was determined macroscopically, and gonads were preserved in formaldehyde – acetic acid – calcium chloride. Individuals with large, yellow gonads possessing visible eggs were classified as ripe females, and those with white, triangular gonads with extractable milt were classified as ripe males. Individuals that had gonads that did not fit either of these descriptions were placed in an "unknown sex" category. Gonads from a random subsample of 80 fish from the total of 200–300 per reef caught by hook and line during the surveys were taken for histological examination and form the basis of this study. Ages of individuals were obtained from counts of sectioned saggitae removed from the fish in the laboratory (e.g., Ferreira and Russ 1994).

Reproductive staging

A validation of the macroscopic staging using histology found the method to be inaccurate, as a large proportion of the samples were reproductively inactive (Adams 1996). Only ripe females and ripe males could be detected accurately in *P. leopardus*. Immature females, mature resting females, transitionals, and newly sex-changed ripe males can all have small, rounded gonads with no specific features to identify them macroscopically (Adams 1996). Accordingly, histological methods were used to reproductively stage all individuals from which this paper was derived (80 fish per reef, $N = 640$). To minimize bias, gonads were processed and examined in random order and without knowledge of the size or age of the fish or the specific reef from which they came. Several gonads ($N = 30$) of both males and females were retained whole and sectioned in the longitudinal and sagittal planes to examine whether gradients in development occurred along or across the gonads. Anterior, middle, and posterior regions of each gonad were examined. No obvious variation in development was observed. Accordingly, the central section of each gonad was removed for histological processing to determine the reproductive stage of the individual. Central portions of each gonad were embedded in paraffin, sectioned at 5-μm thickness, and stained with haematoxylin and eosin–erythrosin (Winsor 1984).

Individuals were classified histologically into developmental categories adapted from Moe (1969) for *Epinephelus morio* (Table 1). Oocyte and spermatogenic stages were adapted from Ferreira (1995) for *P. leopardus*. Female maturity was assessed based on the most advanced oocyte present (West 1990). Mature resting females were defined as those females that had completed spawning for a season. Sexual transition was found to occur in the gonads of prespawning, mature resting females in most cases. Only occasionally was sexual transition evident in the gonads of post-spawned, mature resting females. An individual was classified as a mature male if sexual transition had progressed to the point where the vas deferens was formed and contained spermatozoa, even though ovarian regression was not complete in all instances (Hastings 1981). No categorically "spent" or mature resting males were found, as the majority of males had spermatozoa in their sperm sinuses.

Data analysis

Only the mature individuals ($N = 624$) from the total sample ($N = 640$) were used in analyses. Mature resting females were combined with mature ripe females. Since transitional individuals were functionally neither sex, they were not included in the calculations of sex ratio. Size and age frequency distributions for females, transitionals, and males were constructed from the pooled data from both reefs within each closure status (open and closed to fishing) for each region. Chi-square analyses were used to test for differences in the proportion of males and females above and below the minimum legal size of harvest (38 cm total length (TL), equivalent to 36 cm FL) on open and closed reefs. The age at which 50 and 100% of individuals reached the minimum legal size of harvest (at 36 cm FL) was determined for each region. Fifty and 100% of individuals were above legal size at 3 and 5 years of age, respectively, in the Townsville region and at 3 and 7 years of, respectively, in the Swains region. Chi-square analyses were then used to test for differences in the proportion of males and females above and below each of these ages on open and closed reefs in each region.

Effects of reef closure and regional variation on the sex ratio and average size and age of males, transitionals, and females were analyzed using two-way analyses of variance. Residual plots were examined prior to analyses and data log transformed to satisfy the assumptions of the analysis where necessary. Mean size and age were compared between open and closed reefs and between regions (fixed, orthogonal factors), with reefs as random, replicate management units nested within each region and closure status combination.

Results

Variation in the operational sex ratio between reef closure status and region

The operational sex ratio (mature females to mature males) varied considerably among reefs within each closure status and region (Table 2). In two cases, reefs open and closed to fishing were more similar to each other than to other reefs of the same closure status (Table 2). Nevertheless, average sex ratios differed significantly between regions ($F_{1,4} = 7.377$, $p = 0.05$). Reefs in the Swains region had, on average, a significantly male-biased sex ratio. The highest male bias in the Swains region (0.3) occurred on a reef closed to fishing. In contrast, the sex ratio on the reefs in the Townsville region were female biased, often highly so (e.g., 5.5:1, Townsville open reef) (Table 2). The sex ratio on the Townsville closed reef that exhibited the least female bias (1.3) still differed significantly from unity ($\chi^2 = 10.25$, $p < 0.001$).

Variation in the sex-specific size structure between reef closure status and region

The proportion of mature females that were larger than the minimum legal size for harvest (360 mm FL, 380 mm TL) was significantly greater on closed reefs than on open reefs in both regions (Townsville: $\chi^2 = 8.0$, $p << 0.05$; Swains: $\chi^2 = 23.4$, $p << 0.001$) (Fig. 2). Similarly, a greater proportion of mature males were larger than the minimum legal size on the closed reefs than on the open reefs in the Swains region ($\chi^2 = 27.8$, $p << 0.001$) (Fig. 2*a*). By contrast, proportions of mature males above the legal size limit appeared similar on open and closed reefs in the Townsville region (χ^2 test unreliable because of low expected values) (Fig. 2*b*).

There were significant main effects of closure status and region on the mean size of mature females (Table 3). Mature females were significantly larger on closed reefs than on open reefs (Fig.3) and significantly larger on reefs in the Townsville region than on reefs in the Swains region (Fig. 3). There was also a significant closure status × region interaction effect on the mean size of transitional individuals (Table 3). The average size at sexual transition was significantly lower on closed reefs in the Townsville region than on closed reefs in the Swains region, but the reverse occurred on open reefs (Fig. 3). Further, transitionals on average were smaller on closed reefs than on open reefs in the Townsville region but smaller on the open reefs than the closed reefs in the Swains region. Although the average size of males in the Swains region appeared to be smaller on open reefs than on closed reefs (Fig. 3), effects of reef closure status and (or) region on the average male size were not statistically significant ($p > 0.05$) (Table 3).

Variation in the sex-specific age structure between reef closure status and region

There was a clear effect of reef closure status on female age structure. There were relatively more mature females aged 3 or more years (50% recruited to the fishery) on closed reefs than on open reefs in the Townsville region ($\chi^2 = 18.3$, $p << 0.001$) but not in the Swains region

Table 1. Developmental stages of *P. leopardus* based on histological examination.

Developmental stage	Histological description
Immature female	Ovary dominated by previtellogenic, primary growth stage oocytes (stages 1 and 2). Thin gonad wall.[a] No evidence of prior spawning in the form of postovulatory follicles, atretic cortical alveolus, and (or) vitellogenic (stage 3 and 4) oocytes, brown bodies, or intralamellar muscle bundles[b] in the gonad.
Mature resting female (prespawning and postspawning)	Ovary dominated by previtellogenic, primary growth oocytes (stages 1 and 2) with a thick gonad wall[b] (prespawning). Evidence of recent prior spawning indicated by the presence of postovulatory follicles, atretic stage 3–4 oocytes, and (or) brown bodies (postspawning). Intralamellar muscle bundles[b] can be present in both prespawning and postspawning mature resting females.
Mature ripe/running female	Ovary in active vitellogenesis. Oocytes present in all stages of development; Cortical alveolus (stage 3) and vitellogenic (stage 4) oocytes dominate the ovary. Hydrated (stage 5) oocytes present in ripe/running females.
Transitional	Degenerating oocyte stages 1–4 in the presence of proliferating testicular tissue[c] where spermatozoa have not yet proceeded to fill the peripheral sperm sinuses[d]. Thin bands of primary and secondary spermatocyte, spermatid, and spermatozoa crypts present and often closely associated with peripheries of gonad wall.
Mature ripe male	Proliferation of testicular tissue has progressed to the point where spermatozoa are present in the peripheral sperm sinuses. Testis in active spermatogenesis where all sperm stages (primary and secondary spermatocytes, spermatids, and spermatozoa) are present. Remnant ovarian central cavity visible in all males.

Note: Reproductive criteria adapted from Moe (1969).

[a] Adapted from Burton et al. (1997).

[b] Adapted from Shapiro et al. (1993).

[c] Adapted from Sadovy and Shapiro (1987).

[d] Adapted from Hastings (1981).

Table 2. Sex ratios for each reef within the Townsville and Swains regions. Sex ratios are expressed as females to males and listed in order of increasing male bias.

Heavily female biased → heavily male biased								
Sex ratio	5.5	**2.7**	**2.6**	2.4	1.3	**0.7**	**0.7**	0.3
Closure status	Open	Closed	Open	Open	Closed	Closed	Open	Closed
Region	Townsville	Townsville	Townsville	Swains	Townsville	Swains	Swains	Swains

Note: Bold values indicate reefs that had sex ratios similar to those next to them in the table. Results of test: region, $F_{1,4} = 7.377$, $p = 0.05$; closure status, $F_{1,4} = 3.644$, $p = 0.129$; region × closure status, $F_{1,4} = 0.137$, $p = 0.730$.

(Fig. 4). A significantly greater proportion of mature females were aged 5 years or older (Townsville region) and 7 years or older (Swains region) (100% recruited) on closed reefs than on open reefs (Townsville: $\chi^2 = 51.6$, $p << 0.001$; Swains: $\chi^2 = 13.9$, $p << 0.001$) (Fig. 4).

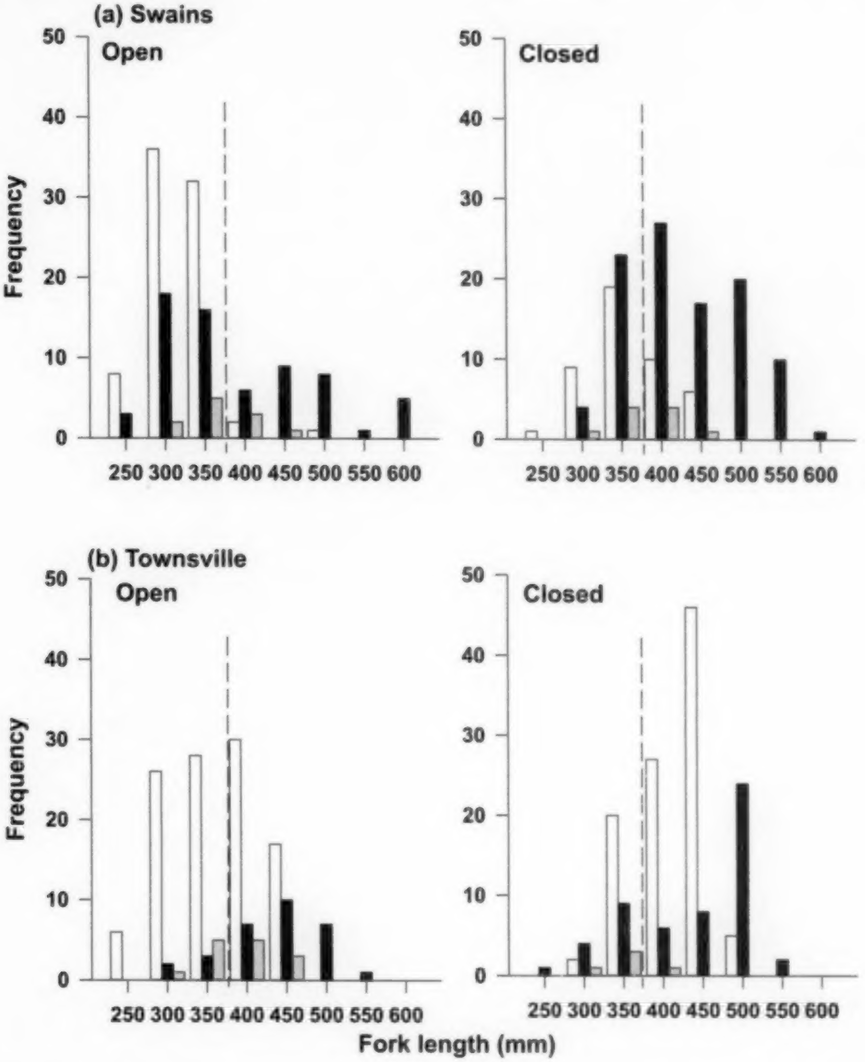
There was little evidence of effects of reef closure status on the male age frequencies in the Swains region (proportion >3 years old: χ^2 test unreliable because of low expected values; proportion >7 years old: $\chi^2 = 0.6$, $p = 0.44$) (Fig. 4*a*). However, there was a marked difference in the age structures of males between open and closed reefs in the Townsville region, where there was a significantly greater proportion of mature males over 5 years of age on closed reefs than on open reefs ($\chi^2 = 5.0$, $p = 0.03$) (Fig. 4*b*). There was no evidence of effects on males over 3 years of age (χ^2 test unreliable because of low expected values).

The average female age was significantly higher on closed reefs than on open reefs in both regions (Table 4; Fig. 5). There was a significant interaction between reef closure status and region affecting the mean age of sexual transition (Table 4). The mean age at sex change was significantly higher on closed reefs than on open reefs in the Swains region but was similar on both closed and open reefs in the Townsville region (Fig. 5). Although male *P. leopardus* on Townsville open reefs appeared to be, on average, younger than those on all the other reefs (Fig. 5), no effects were found to be statistically significant ($p > 0.05$) (Table 4).

Discussion

The patterns of variation in the operational sex ratio and sex-specific size and age structure of *P. leopardus* populations across reef closure management regimes were complex. Differences between open and closed reefs were not consistently expressed as a reduction in the average male size/age or a highly female biased sex ratio, as might have been expected if the effect of closure was to protect local populations from exploitation that disproportionately removes larger fish. Marked regional variation in reproductive biology further complicated the relationship between reef closure and the examined reproductive parameters of *P. leopardus* populations. For example, the effectiveness of reef closures in maintaining a relatively larger average size at sex change and greater proportion of males above the minimum size (and age) of harvest appeared to vary with re-

Fig. 2. Sex-specific frequency distributions of *P. leopardus* populations on open and closed reefs in the (*a*) Swains and (*b*) Townsville regions (open bars, mature females; grey bars, transitionals; black bars, mature males; broken line, minimum legal size (38 cm TL, approximately 36 cm FL)).



gion. Female size and age structure showed the greatest difference between open and closed reefs.

Potential for artifacts of sampling

It might be hypothesized that the regional differences that we observed were artifacts of sampling the regions in different months in different parts of the spawning season. For example, differential movement/distribution of males and females during the different spawning periods has been documented for North Atlantic epinepheline serranids (e.g., Shapiro et al. 1994; Coleman et al. 1996), and sampling at different times or depth strata would be expected to produce different results in such a case. However, spawning aggregations of *P. leopardus* are small (37–75 fish·km^{-2}) relative to North Atlantic epinepheline serranids and are localized in several areas within each reef (Samoilys 1997). There is minimal movement to and from aggregations (hundreds to thousands of metres) within individual reefs (Davies 1995; Zeller 1998; Zeller and Russ 1998) and no evidence of movement among reefs (Davies 1995). Accordingly, the depth-stratified, highly structured sampling at the reef level would have accounted for any fine-scale differential movement between the sexes and effectively targeted the complete reproducing population, irrespective of any within-reef movements of individuals. In addition, sampling was specifically structured to be during the full moon in both regions during the spawning season to avoid the influence of aggregating spawning behavior on our results (Davies et al. 1998). There is also no evidence to suggest that males and females of *P. leopardus* have different distributions or

Table 3. Two-way analysis of variance comparing the mean size of females, transitionals, and males between regions, closure status, and reefs nested within each region and closure status combination.

Source of variation	df	MS	*F*	*p*
Females				
Region	1	5050.73	17.00	**0.015**
Closure	1	5013.51	16.88	**0.015**
Region × closure	1	0.95	<0.01	0.958
Reefs (R, C)	4	297.05		
Transitionals				
Region	1	170.17	1.44	0.296
Closure	1	7.76	0.07	0.810
Region × closure	1	1811.09	15.37	**0.017**
Reefs (R, C)	4	117.84		
Males				
Region	1	3.66	<0.01	0.957
Closure	1	540.19	0.48	0.525
Region × closure	1	44.26	0.04	0.852
Reefs (R, C)	4	1115.98		

Note: Region, closure status, and region × closure status effects were tested against the reef (R, C) MS. Significant *p* values ($p < 0.05$) are indicated in bold.

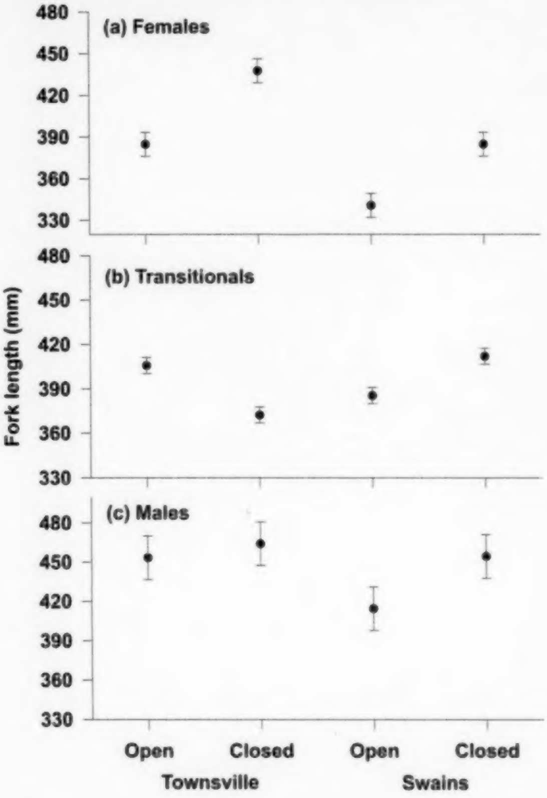
movement patterns when not spawning (Zeller and Russ 1998). We consider it unlikely, therefore, that the patterns that we report were due to artifacts of sampling or regional variation in within-reef movements of individuals.

Effects of reef closures

The effectiveness of reef closures in maintaining a larger, older male population was inconclusive. Males were proportionately more abundant above the minimum legal size on closed reefs in the Swains region but not on closed reefs off Townsville. Alternatively, males were more abundant above the age of 100% recruitment to the fishery on closed reefs only off Townsville. These differences in the proportion of males above the size and age of minimum harvest in the Swains and Townsville regions, respectively, did not translate into statistically significant differences in average size and age. This result probably arose because of the wide range of ages and sizes of male fish in both regions and the consequent high variation around mean size and age. The overall pattern is not consistent with the predictions of contemporary theories on the response of protogynous populations to fishing pressure: a reduced average size and age of males via earlier sex change (compensatory response) or a highly female-biased sex ratio (noncompensating response) (Bannerot et al. 1987). The complex relationship between male size and age and reef closure status may reflect great plasticity in the sexual strategies associated with sex change and their variation between regional populations.

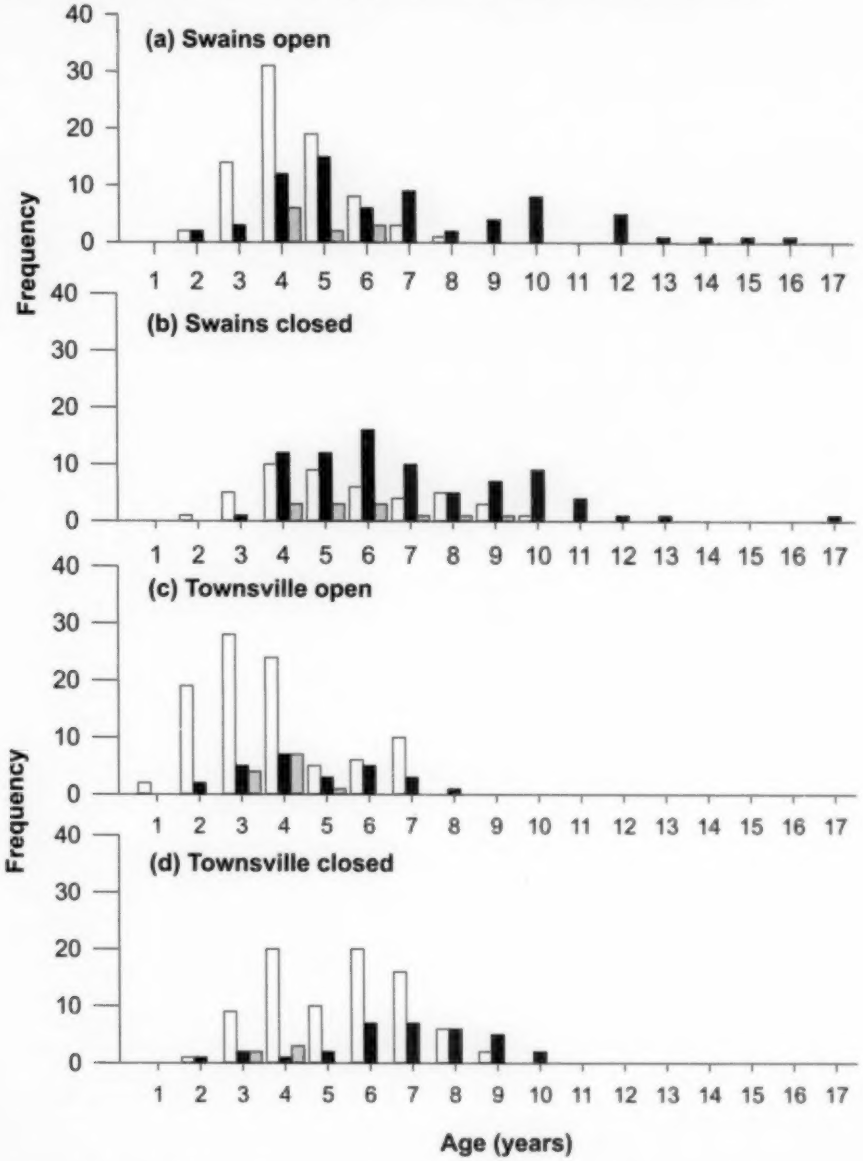
The lack of a significant effect on the mean size and age of males may have been due to growth compensation (accelerated growth) in response to released resources due to lower densities of larger individuals on open reefs (Rothschild 1986). However, it would be expected that both males and females on open reefs would display accelerated growth in response to released resources, rather than males alone. Differential response to resource availability would arise only if male and female *P. leopardus* had sex-specific growth plasticity, such as that suggested for some protogynous Scaridae (Choat et al. 1996) and Sparidae (Garratt et al. 1993). Accelerated growth following sex change could have masked any differences in average male size between open and closed areas as well as resulting in a reduction of older and larger females on open reefs (via earlier sex change). This would explain why males on open and closed reefs in the Townsville region were of similar size yet substantially younger on the open reefs than on the closed reefs. Such flexibility in male development and growth in protogynous populations would make average size and (or) age of males alone a poor indicator of effects of fishing.

Fig. 3. Mean FL (±SE) of *P. leopardus* (*a*) females, (*b*) transitionals, and (*c*) males on open and closed reefs within each region.



The importance of female demography in stock assessment has been well recognized (Huntsman and Schaaf 1994; Sadovy 1996). However, the discussion and examination of effects of fishing on female size and age structure for protogynous hermaphrodites are rare (e.g., Vincent and Sadovy 1998). Females were proportionately more abundant above the minimum legal size and age at recruitment as well as significantly larger and older on closed reefs in both regions in this study. If these patterns were the result of reduced harvest on closed reefs, they suggest that a fishing effect on protogynous hermaphrodites may be detected more readily in the female size and age structure than in male demographics. This may be because the size and age structure becomes more labile with the commencement of sexual transition and

Fig. 4. Sex-specific age frequency distributions of *P. leopardus* populations on Swains (*a*) open and (*b*) closed reefs and Townsville (*c*) open and (*d*) closed reefs (open bars, mature females; grey bars, transitionals; black bars, mature males).



male development. Assuming that fishing effort is considerably higher on open reefs than on closed reefs, the reduced female size and age could be due to two processes: (*i*) the direct removal of females and (or) (*ii*) the indirect loss of females through a compensatory response to reduced male density resulting from fishing (Shepherd and Idoine 1993). In either case, the reduced female size and age on open reefs has the potential to detrimentally affect the reproductive output of the stock. The exponential relationship between fish size and number of eggs produced predicts that the removal of large females by fishing, either directly or through forcing earlier sex change, will reduce the total fecundity of the population (Sadovy 1996). Reef closures, therefore, have the potential to be an effective management strategy against fecundity limitation by maintaining larger females in protected populations.

Regional variation

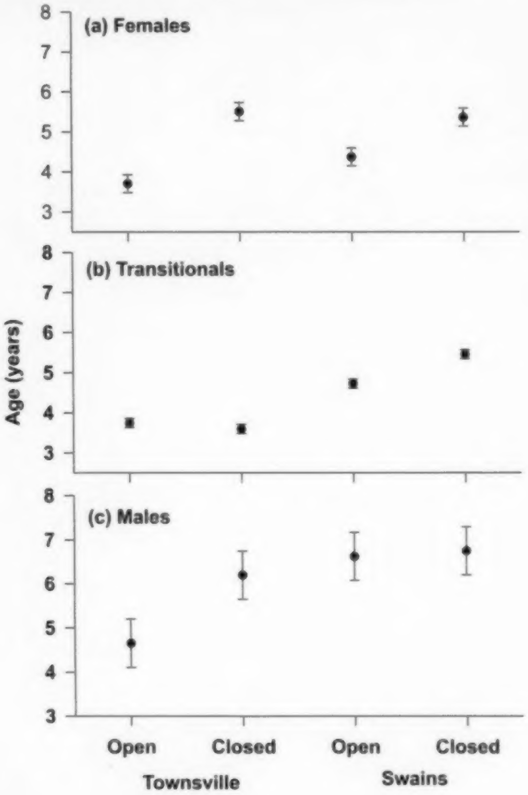
Pronounced regional variation was seen in the sex ratio and sex-specific size and age structure of *P. leopardus* populations. Females were, on average, smaller and individuals changed sex at a significantly older age on the Swains reefs than off Townsville. Most strikingly, populations had a tendency to be male biased in the Swains region. Prevalence of

Table 4. Two-way analysis of variance comparing the mean age of females, transitionals, and males between regions, reef zones, and reefs nested within each region and zone combination.

Source of variation	df	MS	*F*	*p*
Females				
Region	1	0.15	0.76	0.432
Closure	1	3.98	20.25	**0.011**
Region × closure	1	0.29	1.47	0.292
Reefs (R, C)	4	0.20		
Transitionals				
Region	1	3.81	78.38	0.001
Closure	1	0.21	4.34	0.106
Region × closure	1	0.44	9.08	**0.039**
Reefs (R, C)	4	0.05		
Males				
Region	1	3.84	3.21	0.148
Closure	1	2.20	1.84	0.246
Region × closure	1	2.99	2.50	0.189
Reefs (R, C)	4	1.20		

Note: Region, closure status, and region × closure status effects were tested against the reef (R, C) MS. Significant *p* values ($p < 0.05$) are indicated in bold.

Fig. 5. Mean age (±SE) of *P. leopardus* (*a*) females, (*b*) transitionals, and (*c*) males on open and closed reefs within each region.



male-biased sex ratios in the Swains region was an unexpected result. Recent evidence suggests that this is a natural phenomenon, as it has persisted over 4 years in spawning and nonspawning populations (S. Adams, unpublished data). Epinepheline serranids typically exist in female-biased sex ratios (e.g., Moe 1969; Shapiro 1987), although "anomalous" male-biased sex ratios (e.g., 0.13) have been found in *Plectopomus areolatus* in Palau (Johannes et al. 1996, 1994). This was suggested to be the result of differential fishing pressure on males and females in the nonspawning periods. Fishing was focused on inshore areas where females were predominant, resulting in an effective selectivity for the smaller female fish that favored inshore areas (Johannes et al. 1996). There has been a substantial increase in the incentive of the Great Barrier Reef line fishery to target smaller, potentially female, individuals for the live fish export trade. However, this practice commenced only in 1994 and not at all in the Swains region until 1996 (B.D. Mapstone, unpublished data). Therefore, it seems unlikely that the male-biased sex ratios in the Swains region would be explained by preferential targeting of smaller fish.

Slightly male-biased sex ratios have been found in *Epinephelus striatus* and *Mycteroperca microlepis* (Thompson and Munro 1983). *Mycteroperca microlepis*, like many epinepheline serranids, are protogynous with only one male developmental pathway (Shapiro 1987). However, there is evidence to suggest that *E. striatus* is not a monandric protogyne but either secondarily gonochoristic or diandric (Sadovy and Colin 1995). Diandry is a strategy where some males develop directly as males from the juvenile phase (primary males), while others are derived from mature females via sex change (secondary males) (Warner 1984). Diandric protogynes have the potential to form male-biased sex ratios, since primary males can attain large numbers in the population (Warner 1995). The presence of many small males together with older transitional individuals suggests that southern *P. leopardus* populations may have been utilizing an alternative reproductive strategy involving two male pathways. Functional primary males may develop in *P. leopardus* via prematurational sex change, whereby juveniles pass through an initial (nonfunctional) ovarian phase and mature as males. Low numbers of prematurational males have been previously suggested in *P. leopardus*, largely based on their small size (Ferreira 1995). Positive identification of prematurational sex-change males using histology is difficult, as the juvenile female lumen and peripheral sperm ducts are retained in the adult testis (Sadovy and Shapiro 1987). Thus, prematurational males could not be conclusively distinguished from secondary males in this study. However, the existence of such a strategy in the Swains region could explain, in part, the unexpected results in the variation in male size and age structure across reef closure status between regions. Further work is nessessary to demonstrate whether diandry is occurring in these populations.

In conclusion, the inclusion of a broad spatial scale and replication, within units of reef closure status, has demonstrated that the natural variation in reproductive parameters of protogynous populations is considerable. The examined populations on reefs open to exploitation did not show a consistent response across sexes in size and age structure, such as would be expected from traditional theories on exploited protogynous serranids. Despite the marked regional variation, however, reef closures appeared to be an effective management strategy for maintaining, on average, a significantly larger, older female population. Although largely ignored in the past, female size and age structure was shown to be more useful than the traditionally used sex ratio and male/transitional size structure when examining the effects of management zone, and perhaps fishing, on protogynous populations. Controlled, manipulative experiments and the detailed investigation of intra- and inter-specific differences in reproductive patterns will be necessary to resolve further the complexities associated with the response of protogynous populations to fishing pressure and management strategies.

Acknowledgments

We acknowledge financial support from the Cooperative Research Centre for the Ecologically Sustainable Development of the Great Barrier Reef, the Fisheries Research and Development Corporation, the Great Barrier Reef Marine Park Authority, and the Reef Research Augmentative Grant Scheme. The cooperation of Robin Stewart and Mary Petersen, as well as fishers aboard the *Perri*, is gratefully acknowledged. Leigh Winsor gave helpful advice on histological preparation and methods. Otoliths required for aging were processed by D.C. Lou, G.R. Russ, D. Wilson, and S. Slade. This manuscript is a contribution from the CRC Reef Effects of Line Fishing Project.

References

Adams, S. 1996. The effects of reef zoning and region on the sexual structure of *Plectropomus leopardus* populations on the Great Barrier Reef. Honours thesis, James Cook University of North Queensland, Townsville, Australia.

Bannerot, S., Fox, W.W., Jr., and Powers, J.E. 1987. Reproductive strategies and management of snappers and groupers in the Gulf of Mexico and Caribbean. *In* Tropical snappers and groupers: biology and fisheries management. *Edited by* J.Y. Polovina and S. Ralston. Westview Press, Boulder, Co., and London, U.K. pp. 561–603.

Burton, M.P.M., Penney, R.M., and Biddiscombe, S. 1997. Time course of gametogenesis in Northwest Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 122–131.

Choat, J.H., Axe, L.M., and Lou, D.C. 1996. Growth and longevity in fishes of the family Scaridae. Mar. Ecol. Prog. Ser. **145**: 33–41.

Coleman, F.C., Koenig, C.C., and Collins, A.L. 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fishes, **47**: 129–141.

Davies, C.R. 1995. Patterns of movement of three species of coral reef fish on the Great Barrier Reef. Ph.D. thesis, Department of Marine Biology, James Cook University. (Available from Library, James Cook University, Townsville, Queensland, Australia 4811.)

Davies, C.R., Mapstone, B.D., Ayling, A., Lou, D.C., Punt, A., Russ, G.R., Samoilys, M.A., Smith, A.D.M., Welch, D.J., and Williams, McB. 1998. Effects of line fishing experiment 1995–1997: project structure and operations. CRC Reef Res. Cent. Tech. Rep. (Available from the CRC Reef Research Centre, c/o James Cook University, Townsville, Queensland, Australia 4811.)

Ferreira, B.P. 1995. Reproduction of the common coral trout *Plectropmus leopardus* (Serranidae: Epinephelidae) from the central and northern Great Barrier Reef, Australia. Bull. Mar. Sci. **56**: 653–669.

Ferreira, B.P., and Russ, R.G. 1994. Age validation and estimation of growth rate of the coral trout, *Plectropomus leopardus* (Lacepede 1802), from Lizard Island, Northern Great Barrier Reef. Fish. Bull. U.S. **92**: 46–57.

Ferreira, B.P., and Russ, G.R. 1995. Population structure of the leopard coral grouper, *Plectropomus leopardus*, on fished and unfished reefs off Townsville, Central Great Barrier Reef, Australia. Fish. Bull. U.S. **93**: 629–642.

Fulton, E.A.S. 1996. An investigation of the determinants of the commercial catch rate of *Plectropomus leopardus* (Serranidae) with regard to the targeting of spawning aggregations, using computer simulations. Honours thesis, Department of Marine Biology, James Cook University. (Available from Library, James Cook University, Townsville, Queensland, Australia 4811.)

Garratt, P.A., Govender, A., and Punt, A.E. 1993. Growth acceleration at sex change in the protogynous hermaphrodite *Chrysoblephus puniceus* (Pisces: Sparidae). S. Afr. J. Mar. Sci. **13**: 187–193.

Hastings, P.A. 1981. Gonad morphology and sex succession in the protogynous hermaphrodite *Hemianthias vivianus* (Jordan and Swain). J. Fish Biol. **18**: 443–454.

Heemstra, P.C., and Randall, J.E. 1993. Groupers of the world: FAO species catalogue. FAO Fish. Synop. **16**(125): 1–11.

Huntsman, G.R., and Schaaf, W.E. 1994. Simulation of the impact of fishing on the reproduction of a protogynous grouper, the graysby. N. Am. J. Fish. Manage. **14**: 41–52.

Jennings, S., and Lock, J.M. 1996. Population and ecosystem effects of reef fishing. *In* Reef fisheries. *Edited by* N.V.C. Polunin and C.M. Roberts. Chapman and Hall, London, U.K. pp. 193–218.

Johannes, R.E., Squire, L., and Graham, T. 1994. Developing a protocol for monitoring spawning aggregations of Palauan serranids to facilitate the formulation and evaluation of strategies for their management. Prog. Rep. Sth. Pac. Forum Fish. Agency, Honiara, Solomon Islands.

Johannes, B., Squire, L., Graham, T., Renguul, H., and Bukurrou, A. 1996. Palau grouper spawning aggregation research project 1995 progress report. Prog. Rep. Forum Fish. Agency, Honiara, Solomon Islands.

Mapstone, B.D., Mckenlay, J.P, and Davies, C.R. 1996*a*. A description of the commercial reef line fishery log book data held by the Queensland Fisheries Management Authority. Report to the Queensland Fisheries Management Authority, Brisbane. (Available from the Queensland Fisheries Management Authority, P.O. Box 344, Fortitude Valley, Queensland, Australia 4006.)

Mapstone, B.D., Campbell, R.A., and Smith, A.D.M. 1996*b*. Design of experimental investigations of effects of line and spear fishing on the Great Barrier Reef. CRC Reef Res. Cent. Tech. Rep. No. 7. (Available from the CRC Reef Research Centre, c/o James Cook University, Townsville, Queensland, Australia 4811.)

Mapstone, B.D., Davies, C.R., Lou, D.C., Punt, A.E., Russ, G.R., Ryan, D.A.J., Smith, A.D.M., and Williams, McB. 1998. Effects of line fishing experiment 1995–1997: progress report. CRC Reef Res. Cent. (Available from the CRC Reef Research Centre, c/o James Cook University, Townsville, Queensland, Australia 4811.)

Moe, M.A., Jr. 1969. Biology of the red grouper *Epinephelus morio* (Valenciennes) from the eastern Gulf of Mexico. Fla. Dep. Nat. Resour. Mar. Lab. Prof. Ser. No. 10.

Punt, A.E., Garrat, P.A., and Governder, A. 1993. On an approach for applying per-recruit methods to a protogynous hermaphrodite with an illustration for the slinger, *Chrysoblephus puniceus* (Pisces: Sparidae). S. Afr. J. Mar. Sci. **13**: 109–119.

Rothschild, B.J. 1986. Dynamics of marine fish populations. Harvard University Press, Cambridge, Mass.

Russ, G.R., Lou, D.C., and Ferreira, B.P. 1995. A long term study on population structure of coral trout on reefs open and closed to fishing in the central Great Barrier Reef. CRC Reef Res. Cent. Tech. Rep. No. 3. CRC Reef Research Centre, Townsville, Australia.

Russ, G.R., Lou, J.B., and Ferreira, B.P. 1998. Mortality rate of a cohort of the coral trout, *Plectropomus leopardus*, in zones of the Great Barrier Reef Marine Park closed to fishing. Mar. Freshwater Res. **49**: 507–511.

Sadovy, Y. 1996. Reproduction of reef fishery species. *In* Reef fisheries. *Edited by* N.V.C. Polunin and C.M. Roberts. Chapman and Hall, London, U.K. pp. 15–59.

Sadovy, Y., and Colin, P.L. 1995. Sexual development and sexuality in the Nassau grouper. J. Fish Biol. **46**: 961–976.

Sadovy, Y., and Shapiro, D.Y. 1987. Criteria for the diagnosis of hermaphroditism in fishes. Copeia, **1987**: 136–156.

Samoilys, M.A. 1997. Periodicity of spawning aggregations of coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the northern Great Barrier Reef. Mar. Ecol. Prog. Ser. **160**: 149–159.

Shapiro, D.Y. 1981. Size, maturation, and social control of sex reversal in the coral reef fish *Anthias squamipinnis*. J. Zool. (Lond.), **193**: 105–128.

Shapiro, D.Y. 1987. Reproduction in groupers. *In* Tropical snappers and groupers: biology and fisheries management. *Edited by* J.Y. Polovina and S. Ralston. Westview Press, Inc., Boulder, Co. pp. 295–327.

Shapiro, D.Y., Sadovy, Y., and McGehee, A. 1993. Periodicity of sex change and reproduction in the red hind, *Epinephelus guttatus*, a protogynous grouper. Bull. Mar. Sci. **53**: 1151–1162.

Shapiro, D.Y., Garcia-Molina, G., and Sadovy, Y. 1994. Social system of an inshore stock of the red hind grouper, *Epinephelus guttatus* (Pisces: Serranidae). Environ. Biol. Fishes, **41**: 415–422.

Shepherd, B.R., and Idoine, J.S. 1993 Length-based analysis of yield ad spawning biomass per recruit for black sea bass *Centropristis striata*, a protogynous hermaphrodite. Fish. Bull. U.S. **91**: 328–337.

Thompson, R., and Munro, J.L. 1983. The biology, ecology and bionomics of the hinds and groupers, Serranidae. *In* Caribbean coral reef fishery resources. *Edited by* J.L. Munro. ICLARM Stud. Rev. 7. ICLARM, Manila. pp. 59–81.

Vincent, A.C.J., and Sadovy, Y.J. 1998. Reproductive ecology in the conservation and management of fishes. *In* Behavioural ecology and conservation biology. *Edited by* T.M. Caro. Oxford University Press, New York. pp. 209–245.

Warner, R.R. 1984. Mating behavior and hermaphroditism in coral reef fishes. Am. Sci. **72**: 128–136.

Warner, R.R. 1995. Large mating aggregations and daily long-distance spawning migrations in the blue head wrasse, *Thalassoma bifasciatum*. Environ. Biol. Fishes, **44**: 337–345.

West, G. 1990. Methods of assessing ovarian development in fishes: a review. Aust. J. Freshwater Res. **41**: 199–222.

Winsor, L. 1984. Manual of basic zoological microtechniques for light microscopy. James Cook University Library, James Cook University Library, Townsville, Australia.

Zeller, D.C. 1998. Spawning aggregations: patterns of movement of the coral trout *Plectropomus leopardus* (Serranidae) as determined by ultrasonic telemetry. Mar. Ecol. Prog. Ser. **162**: 253–263.

Zeller, D.C., and Russ, G.R. 1998. Marine reserves: patterns of adult movement of the coral trout *Plectropomus leopardus* (Serranidae). Can. J. Fish. Aquat. Sci. **55**: 917–924.

Using perceptions of data accuracy and empirical weighting of information: assessment of a recreational fish population

Margaret F. Merritt and Terrance J. Quinn, II

Abstract: Recreational fisheries management is often compromised by limited information of variable quality from several sources. We develop a form of catch-age analysis to combine uncertain information from creel surveys, age composition, and mark–recapture estimates of abundance. Four systems are used in weighting annual observations: equal, inverse of squared coefficients of variation (CV^{-2}), perceptions of accuracy, and a combination of the latter two. The model is applied to a humpback whitefish (*Coregonus pidschian*) population in Alaska and evaluated for model fit, parameter uncertainty, conservative forecasts of exploitable abundance, and biological plausibility. The probability of forecasted stock abundance occurring below a threshold level defined by an agency management plan is evaluated for various recruitment and exploitation scenarios. The perception model is judged to be best with the use of the analytic hierarchy process, a decision-making technique. By incorporating perceptions into fisheries decision-making, beliefs in the accuracy of uncertain information are made explicit. In a conservative context, fishery management decisions should include reducing risk to the stock in the setting of harvest policy and in the selection of the assessment model.

Résumé : La gestion des pêches sportives est souvent compromise par le fait que l'information dont on dispose est limitée, de qualité variable, et issue de sources diverses. Nous avons mis au point un mode d'analyse de l'âge à la capture pour combiner l'information incertaine fournie par les enquêtes auprès des pêcheurs, la composition par âge et les estimations de l'abondance par marquage–recapture. Nous avons pondéré les observations annuelles à l'aide de quatre systèmes : égalité, inverse des coefficients de variation carrée (CV^{-2}), perceptions de l'exactitude, et enfin une combinaison des deux derniers. Le modèle est appliqué à une population de corégone à bosse (*Coregonus pidschian*) de l'Alaska, et nous avons évalué l'ajustement du modèle, l'incertitude des paramètres, les prévisions prudentes de l'abondance exploitable et la plausibilité biologique. Nous avons évalué la probabilité de voir l'abondance prévue du stock tomber au-dessous d'un seuil défini par le plan d'un organisme de gestion pour divers scénarios de recrutement et d'exploitation. C'est le modèle sur les perceptions qui est jugé le meilleur, employé avec la méthode décisionnelle de hiérarchie multicritère. En intégrant les perceptions dans la prise de décision sur les pêches, on rend explicite les croyances sur l'exactitude de l'information incertaine. Dans un contexte de gestion prudente, les décisions de gestion des pêches doivent inclure la réduction du risque pour le stock dans l'établissement de la politique de prélèvement et dans le choix du modèle d'évaluation.

[Traduit par la Rédaction]

Introduction

Fisheries management is a decision-making process in which managers must often work with uncertain parameters in models that may inadequately describe the process and with information of unknown precision and accuracy. Because the sources of uncertainty will never be completely removed, fisheries managers have personal perceptions about various sources of information and the plausibility of estimates, which routinely leads to intuitive judgments in their decision-making. Perception is a type of inference that occurs when mental images are converted into interpretations, resulting in beliefs. Perceptions are particularly important in situations where there is little current knowledge, yet decisions must be made on the basis of best available information.

While there is no question that perceptions play a role in decision-making (Tait 1988; Badinelli and Baker 1990) and perceptions are important to fisheries management decisions (Pearse and Walters 1992), little research has been conducted to quantify the influence of perceptions on solutions to a fisheries problem. Martin (1979) used subjective probability estimates to determine the optimum number of units of gear in salmon drift gillnet fisheries. Geiger and Koenings (1991) incorporated subjective information about spawner–recruit parameters based on perception of smolt characteristics and lake system limitations into a Bayesian framework to evaluate escapement goals for sockeye salmon (*Oncorhynchus nerka*). Weighting according to perceptions is not commonly found in abundance estimation procedures, but it

Received July 16, 1999. Accepted March 2, 2000.
J15253

M.F. Merritt.[1] Alaska Department of Fish and Game, Sport Fish Division, 1300 College Rd., Fairbanks, AK 99701, U.S.A.
T.J. Quinn, II. Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 11120 Glacier Hwy., Juneau, AK 99801-8677, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: peggy-merritt@fishgame.state.ak.us

is employed in decision analysis (see Srinivasan and Shocker 1973).

An alternative way of incorporating uncertainty into decision-making is to use models that explicitly weight by the inverse of the variance, or equivalently the statistical information, of the data. Inverse variance weighting is commonly employed in weighted regression analysis (Steel and Torrie 1960) and in abundance estimation (following Paloheimo, cited in Seber 1982) when observations have unequal variances. In weighted regression models the inverse variances are used as weights in the residual sum of squares (Abraham and Ledolter 1983), and inverse variance weighting usually leads to smaller variances in parameter estimates.

This study examines the influence of perceptions and empirical inverse variances (expressed in relative terms as the inverse of squared coefficients of variation, or CV^{-2}) on the performance of a catch-age model. Perception used here refers to a belief in the accuracy of information. Where harvest can be sufficiently sampled for age structure and auxiliary information is available, catch-age analysis (Deriso et al. 1985; Funk et al. 1992) can be a useful analytical tool for modeling the interaction of the fish stock with a recreational fishery. We use this tool with the humpback whitefish (*Coregonus pidschian*) population in the Chatanika River, Alaska, which is subject to a recreational spear fishery. The goal is to account for risk in the threshold harvest management of humpback whitefish as specified by an agency management plan described below. One common measure of risk in fisheries management involves the probability of an undesirable event (e.g., stock abundance falling to an unacceptable level) leading to some consequence or loss (e.g., diminished social or economic benefits derived from fishing) in relation to a reference point (Francis and Shotton 1997).

Four systems for weighting annual observations are examined: equal (hereafter referred to as the base model), CV^{-2}, perceptions of accuracy by a knowledgeable individual, and a combination of perception and CV^{-2} (hereafter referred to as the combined model). Equal weighting of data has often been used in catch-age analysis (Funk et al. 1992; Zheng et al. 1993).

Model performance is judged using the analytic hierarchy process (Saaty 1990), a decision-making technique that leads to a "best" model. Four criteria are identified to judge model performance: model fit of the data sets, parameter uncertainty, conservative forecasts of exploitable abundance, and biological plausibility of estimates.

Conservatism in setting harvest policy has been advocated when there is uncertainty (Clark 1985), but to incorporate conservatism in the stock assessment method is a concept that is now receiving more attention. Management agencies often have competing and uncertain assessments, in which they usually choose the more conservative one. Thus, a model with a low probability of dropping below a specified abundance level would be considered less conservative than one with a higher probability. The level of acceptable risk is implicit in the assessment that is most preferred (Saaty 1990). Thus, fisheries managers express their level of risk acceptance in setting harvest policy *and* in selecting the stock assessment method.

We also use the models to assess the probability of stock abundance declining below a threshold level in the harvest policy by forecasting exploitable abundance for varying exploitation and recruitment scenarios using age-structured models. Here, a harvest policy with a lower probability of dropping below a prespecified abundance level would be considered more conservative than one with a higher probability.

Materials and methods

Approach

Information from a typical recreational fishery includes (*i*) total harvest, (*ii*) age composition, and (*iii*) a survey or index of exploitable abundance. In the Application section, we illustrate how uncertainties in this information can be modeled. We then describe how these sources of information can be combined into an age-structured model to provide estimates of abundance, age vulnerability, and exploitation rate. Annual weights for the data sources come from quantifying a manager's perceptions of data accuracy or incorporating a CV^{-2} scheme.

Data

Total harvest, H_y, in year y and its variance $V[H_y]$ are usually estimated using standard sampling survey techniques (e.g., Thompson 1992) by extrapolating sample harvest to total harvest. Age composition is usually obtained from a simple random sample or a two-stage length–age protocol (Quinn and Deriso 1999). For simple random sampling, age samples of size n_y are often assumed to be randomly drawn from a multinomial distribution. The estimated proportion at age a in year y, $\hat{p}_{a,y}$, is then $\hat{p}_{a,y} = n_{a,y}/n_y$, where $n_{a,y}$ is the number of fish of age a in the sample. Estimated harvest at age is then $\hat{H}_{a,y} = \hat{H}_y \hat{p}_{a,y}$, where $\hat{H}_y$ is the estimated harvest from the creel survey in year y.

A survey or fishery index of abundance is obtained from a particular sampling strategy or analytical method. Approaches include catch per unit of effort, transect, mark–recapture, and removal methods (Seber 1982).

Error structure

It is necessary to describe the error structure of the data in order to develop a model. Like Kimura (1990), we prefer to use empirical distributions to model error structure. Creel harvest estimates are often assumed to be normally distributed. The multinomial model for age composition error is assumed (Methot 1990). A common alternative is lognormal error for harvest at age. Additional approaches to modeling the multinomial distribution include adding a dispersion parameter (Fukushima et al. 1998) and modeling observed age composition with the Dirichlet distribution (Williams and Quinn 1998). Survey error structures are often normal or lognormal in distribution; we present an inverse normal distribution in the application.

Annual weights

Many fisheries managers know the circumstances under which data are collected and have subjective perceptions of the data's accuracy. Perceptions can be influenced by observations of experimental design, sampling strategy, environmental conditions under which sampling is conducted, and expertise of personnel involved. These perceptions can be quantified by asking the manager to rate his/her beliefs in the accuracy of annual abundance, harvest, and age composition estimates using a scale between 0 (inaccurate) and 1.0 (accurate) where increments of 0.1 represent differences in degrees of perceived accuracy. By assigning a numerical score to estimates each year, data can be weighted (θ_x) according to their perceived accuracy.

Alternatively, the weights (θ_x) can be chosen inversely proportional to the estimated variation of the data. In practice, we have found that this weighting scheme can effectively give weights near

0 to large amounts of data when the data vary over orders of magnitude (such as catch data). In this situation, it is sometimes better to weight by the inverse of the *relative* variation in the data as measured by the CV^{-2}. (Various process errors could be added; however, we were unable to do so due to the limits of the data.) A combined model for weighting is obtained by multiplying the CV^{-2} and perception weights together for each annual observation and then normalizing the result. A naive approach is to use equal weighting in which each annual estimate has the same weight; this is the most common situation in practice.

Catch-age models

In catch-age analysis, an age-structured population model is developed to estimate catch, age composition, and related information from population parameters for recruitment, gear selectivity, fishing mortality, and catchability (Deriso et al. 1985; Quinn and Deriso 1999). Parameter estimates are often obtained by fitting the model to data using nonlinear least squares or maximum likelihood approaches according to some objective function. Auxiliary information in the form of indices or survey estimates of abundance is needed to stabilize parameter estimates.

Without loss of generality, we minimize the objective function. In the case of our typical recreational fishery, the three objective function components are the harvest sum of squares O_{harvest}, the negative of the log likelihood component for age composition O_{age}, and the sum of squares for the survey index O_{index}. A given O component can be written

$$(1) \qquad O = \lambda \sum_{x} \theta_x O_x$$

where λ is an overall weight used to scale O components, θ_x is a data-specific weight given to O components, O_x is a data-specific sum of squared deviations of observed and estimated values (or related objective), and x is the data index (such as year, age, or some combination).

The θs represent the weight of influence given to a subset of data within an individual O component; in our study, they are determined annually. Scaling coefficients (λ) are weights for the O_xs from different data sources. Theoretically, scaling coefficients are ratios of variances between the catch data and another data source (Quinn and Deriso 1999), although in many situations, these variances are not known, so must be estimated. Alternatively, scaling coefficients can be used to bring the Os closer in magnitude (Merritt 1995) for better parameter estimation or to downweight a contradictory data source.

Forecasting exploitable abundance

Forecasts are used to evaluate population trends, set catch limits, and examine the probability of the population falling below some level. In age-structured models, forecasting is often a three-step process: (1) decide on a harvest rate or fishing mortality to use, (2) project abundance at other ages from the most recent abundance estimates from the model, and (3) forecast recruitments in future years using a deterministic or stochastic model. In step 3, the deterministic model is usually a constant recruitment, density-independent (recruitment proportional to spawning biomass), or spawner–recruit model such as the Ricker or Beverton–Holt. A stochastic model usually combines a deterministic model with a normal or lognormal error structure. Confidence intervals can be developed from the bootstrap distribution of forecasts using the percentile method (Efron and Tibshirani 1993). Alternative recruitment scenarios can be subjected to the same forecasting treatment and the results summarized in a decision table (Hilborn et al. 1994).

Choosing the best model

Model performance can be judged using the following criteria: (*i*) a model should fit the individual data components well with the residual mean square (RMS) measuring the fit of each model component, these fits then being compared pairwise using Lehmann's asymptotic test (Lehmann, cited in Criddle and Havenner 1991); (*ii*) a model should produce parameter estimates with small variance and minimal bias; as a summary measure of bias and variability, we use the root mean square error $\text{RMSE} = \sqrt{\text{SD}^2 + \text{bias}^2}$; (*iii*) forecasts of exploitable abundance should be conservative; we evaluate the probability of forecasted exploitable abundance falling below a threshold level from 1000 bootstrap iterations; and (*iv*) estimates should be plausible and within the realm of productivity of the stock.

As these criteria need not support the same model, a way of synthesizing these considerations is needed. The analytic hierarchy process (Saaty 1990) incorporates quantitative measures and intuitive judgments into a mathematical process used to describe the problem and arrive at a solution. The problem of selecting the best model is structured as a hierarchy, with goal at the top, criteria to be considered in subsidiary levels, and models to be evaluated at the bottom. Weighting of criteria used in judging model performance is accomplished prior to fitting the models using the computer program Expert Choice (Expert Choice (1998), Decision Support Software, Pittsburgh, Pa.). Pairwise judgments of the relative importance of two criteria are expressed using an inverse positive ratio scale devised by Saaty (1990), modified in this paper. The modified scale consists of values 1/5 to 5, with verbal meanings as follows: 1/5 (strongly unimportant or undesirable), 1/3 (moderately unimportant), 1 (equally important), 3 (moderately important), and 5 (strongly important). Compromise judgments are interpolated. A scoring system is then set up and model performance is rated against criteria. The total score for each model is calculated by adding the weighted proportions of all criteria for that model. The total score of each model determines its rank (for details, see Saaty 1990).

Application

The humpback whitefish population in the Chatanika River, Alaska, is subject to a recreational spear fishery (Merritt 1995). To achieve sustained yield, management follows a threshold harvest policy that is likely to reduce the risk of a stock collapse and to enhance long-term productivity (Quinn et al. 1990). The threshold abundance level of 10 000 humpback whitefish was previously determined heuristically, based on the observed range of historical abundances and harvests. Higher exploitation rates are allowed as the population increases: at 10 000 – 15 000 spawners, the allowable maximum harvest rate is 10%; at 15 000 – 20 000 estimated spawners, harvest is held to the "midrange" of between 10 and 15%. When estimated spawner abundance exceeds 20 000 fish, harvest is allowed to approach the 15% exploitation limit.

Previous assessments involved subjective assimilation of the information about harvest, age composition, and abundance. We use catch-age analysis to assimilate the information in order to provide a firmer quantitative basis for the assessment. We incorporate a manager's perceptions about the population, fishery, and historical sampling of the fish stock and harvest directly into the assessment, as described below. Forecasting future age distributions of abundance for alternative scenarios about recruitment allows us to evaluate exploitation rates specified in the management plan, given various states of the population.

Data

Three data sources are used: (*i*) harvest at age estimates from a creel survey (1986–1992 except 1991 when the fishery was closed), (*ii*) estimates of exploitable abundance from mark–recapture experiments (1986–1992 except 1990 when the mark–recapture experiment failed), and (*iii*) observed age frequencies (1986–1992), ages 3 through 10+ (Table 1).

Table 1. Estimates of harvest, exploitable abundance, and proportion by age for humpback whitefish, Chatanika River, 1986–1992.

			Age (years)							
Year	Harvest (SE)[a]	Abundance (SE)[b]	3	4	5	6	7	8	9	10+
1986	2528 (914)	14 906 (3172)	0	0.205	0.356	0.164	0.151	0.096	0.027	0
1987	4577 (926)	28 165 (3434)	0.028	0.290	0.357	0.201	0.079	0.026	0.015	0.004
1988	3572 (293)	41 211 (5155)	0.005	0.156	0.421	0.284	0.099	0.026	0.009	0
1989	3835 (491)	17 322 (1655)	0	0.013	0.215	0.441	0.202	0.086	0.023	0.020
1990	956 (34)	—	0.010	0.050	0.257	0.330	0.221	0.083	0.023	0.025
1991	0	15 313 (2078)	0.029	0.038	0.058	0.149	0.251	0.208	0.129	0.137
1992	393 (9)	20 180 (1633)	0.011	0.064	0.056	0.075	0.184	0.229	0.176	0.204

[a] The fishery was closed in 1991.
[b] No mark–recapture abundance estimate was generated in 1990.

Table 2. Weights of influence (θ) assigned to each annual objective function component (harvest, mark–recapture abundance, and age composition estimates) for the four models used in estimating abundance of humpback whitefish in the Chatanika River.

Year	Harvest[a]	Abundance[b]	Age composition
Base model			
1986	1	1	1
1987	1	1	1
1988	1	1	1
1989	1	1	1
1990	1	0	1
1991	0	1	1
1992	1	1	1
CV^{-2} model			
1986	0.003	0.148	0.018
1987	0.010	0.450	0.516
1988	0.062	0.429	0.367
1989	0.024	0.724	1.000
1990	0.250	0	0.009
1991	0	0.362	0.214
1992	1.000	1.000	0.450
Perception model			
1986	0.6	0.7	0.6
1987	0.7	0.7	0.6
1988	0.8	0.7	0.6
1989	0.6	0.2	0.6
1990	0.9	0	0.6
1991	0	0.8	1.0
1992	0.9	0.8	1.0
Combined model			
1986	0.002	0.130	0.018
1987	0.008	0.394	0.516
1988	0.055	0.375	0.367
1989	0.016	0.181	1.000
1990	0.250	0	0.009
1991	0	0.362	0.357
1992	1.000	1.000	0.750

[a] The fishery was closed in 1991.
[b] No mark–recapture abundance estimate was generated in 1990.

Error structure

In examining the empirical distributions of the three data sources, we found no reason to reject the assumption of normality for creel estimates of harvest or the multinomial assumption for age composition data. The mark–recapture experiment followed the Petersen design of single release, single recapture (Seber 1982). Performing a bootstrap procedure showed that during several years, distributions of abundance are skewed due to low numbers of recaptures. The inverse transformation of the mark–recapture estimates provides a good approximation to normality, in accord with theory (Seber 1982).

Annual weights

To incorporate perceptions about data accuracy, we asked the fishery manager to rate the quality of annual abundance, harvest, and age composition estimates on a scale of 0 to 1. For example, a low score (0.2) was assigned to the 1989 abundance estimate (Table 2) because the manager perceived that the sampling strategy did not meet the assumption of closure in the mark–recapture estimator.

Empirical sampling variances differed interannully due to large changes in the magnitudes of harvest and fish abundance and increasing efficiency in creel survey and mark–recapture study designs over time. The most weight in the harvest data was placed on the estimate in 1992 because its CV was the smallest, compared with prior years' harvest estimates (Table 2). Because the CV^{-2} weighting scheme gave a weight for harvest in 1992 greater in magnitude than in 1986 and 1987, this gave less weight to harvest in earlier years. Similar differences in annual harvest weights occur in the combined model because its weighting scheme is the product of the CV^{-2} and perception weights (Table 2).

Catch-age models

We used an Excel spreadsheet form of the catch-age analysis approach of Deriso et al. (1985), developed in Funk et al. (1992). The specific form of the catch-age model used in this paper is given in Merritt (1995). The four weighting models are applied to the humpback whitefish data set in accord with the principles described above. The overall objective function comprises the sum of squares for the harvest estimates, the negative of the log likelihood multinomial component for age composition, and the sum of squares component for the inverses of exploitable abundance from the mark–recapture experiment.

A bootstrap procedure is used to obtain standard errors for parameter estimates (Efron and Tibshirani 1993), wherein age composition is sampled with replacement from the multinomial distribution, and residuals are resampled and added to predicted values for the harvest and survey components. The bootstrap procedure is repeated 1000 times for each of the four models.

Forecasting exploitable abundance

The four models are used in the forecasting procedure to examine their conservatism and to evaluate the management plan. Starting with estimated abundances by age in 1993 for each bootstrap replication (except for age 3, which must be forecasted), the exploitable abundance is forecasted for years the 1993–1998. Percentile ranges at the 2.5 and 97.5% levels are generated to approximate the amount of forecast variability in total annual exploitable abundance.

Two recruitment scenarios are examined: constant and a density-independent relationship:

$$(2) \qquad R_t = \alpha \mathrm{EN}_{t-3}$$

Constant recruitment is based on a random sample of recruitment during 1986–1992. The density-independent relationship between recruits and spawning stock size is uncertain due to only four data points, but the variation about the relationship is included in the forecast using lognormal error. The constant recruitment scenario is more optimistic than the density-independent one because higher recruitments estimated for 1986–1988 are used (but not in the density-independent scenario because there is no corresponding lagged estimate of exploitable abundance).

Exploitation rate as described in Merritt (1995) is examined at 0, 10, and 15% because these are the average rates of harvest specified in the management plan.

Choosing the best model

In our judgment, first, the model should fit the data sources well, second, one desires parameter estimates that are of low bias and variability, third, estimates of exploitable abundance should be conservative, and fourth, parameter estimates must be plausible. Our judgement matrix is

	Model fit	Uncertainty	Conservatism	Plausibility
Model fit	1	1.5	2.0	2.5
Uncertainty		1	1.5	2.0
Conservatism			1	1.5
Plausibility				1

For example, model fit is 1.5 times as important as uncertainty and 2.0 times as important as conservatism, in our opinion. Although we chose the values based on our beliefs, we have made those beliefs explicit. The normalized eigenvector for weighting criteria is

$$w = \begin{bmatrix} \text{Fit} & 0.386 \\ \text{Uncertainty} & 0.277 \\ \text{Conservatism} & 0.196 \\ \text{Plausibility} & 0.141 \end{bmatrix}$$

The potential values that could be obtained for each criterion are rated for their desirability (Table 3). For example, small RMSs of the model are strongly desirable (=5), whereas large RMSs indicate poor model fit and are strongly undesirable (=1/5).

Results

Estimated parameters

Age-specific gear vulnerability curves show lower vulnerability until older ages (8+ years) for the CV^{-2} and combined models, as compared with the base and perception models. Ages at 50% vulnerability for the CV^{-2} and combined models are 5.3 and 5.1 years, respectively, and 4.7 years for both the base and perception models. As a result, the CV^{-2} and combined models are less conservative because they predict less effect of harvesting.

All models generate plausible estimates of exploitable abundance. Estimated exploitable abundance increases from 15 000 – 18 000 fish in 1986 to 29 000 – 34 000 fish in 1988, similar to the trend observed in mark–recapture experiments (Fig. 1). While exploitable abundance estimated by catch-age models is similar between 1988 and 1989, in contrast, a precipitous decline in exploitable abundance is estimated in mark–recapture experiments. Thus, relative to catch-age models, the mark–recapture experiment underestimated abundance in 1989. Strong recruitment as evidenced in age composition just prior to 1989 (Table 1) argued for a higher abundance estimate than observed in the mark–recapture experiment. The models account for this recruitment (Fig. 1), whereas the mark–recapture experiment in 1989 estimated abundance too low because the assumption of closure was not satisfied. Declines in exploitable abundance estimated by catch-age models occur in 1990–1992.

Table 3. Rating of criterion values.

Value of a criterion	Rating
Model fit: RMS[a]	
Best fit	5
Worst fit (significant at $p < 0.01$)	1/5
Parameter uncertainty: RMSE[b]	
Lowest error (value is <70% of 1.0)	5
Moderate error (value is 70–85% of 1.0)	3
Highest error (value is >85% of 1.0)	1/5
Forecast: EN (p < 10 000 fish) at 15% exploitation[c]	
Conservative, $p \geq 0.15$	5
Moderate, $0.10 < p < 0.15$	3
Optimistic, $p \leq 0.10$	1/5
Plausibility of EN for 1986–1992[d]	
10 000 – 40 000 fish	5
<10 000 fish	1/5
>40 000 fish	1/5

[a] Significant from zero based on Lehman's test.

[b] Total values are normalized among models so that the highest error is assigned 1.0. Model totals are then proportionally compared with 1.0 for each parameter.

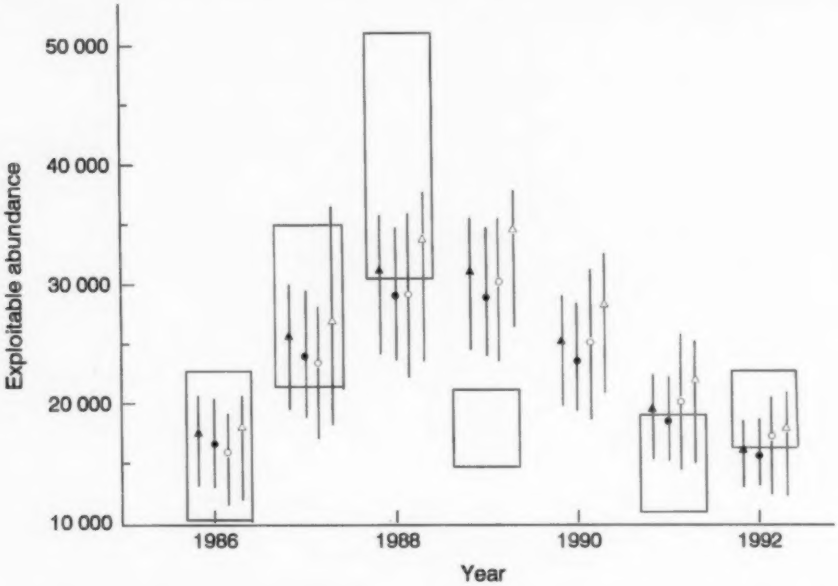
[c] Probability is based on 1000 bootstraps for each of six years (1993–1998) for a total of 6000 chances that EN will be below a threshold of 10 000 fish (see Table 4); the greater the probability, the more conservative the stock assessment model. The constant recruitment scenario is presented.

[d] Expert judgment based on probable productivity of stock and historical performance.

Average exploitation for catch-age models in 1986 is estimated at about 14–16%. The catch-age models suggest that the empirical information slightly underestimates exploitation in 1987 and 1988 and greatly overestimates exploitation in 1989 (because there was no population estimate in 1990, no exploitation rate could be calculated). The discrepancies in average exploitation rates between the empirical data and the catch-age models are due largely to exploitable abundance estimates. For example, the empirical data overestimates average exploitation in 1989 largely due to the underestimation of exploitable abundance by the mark–recapture experiment during that year (see Fig. 1).

Strong age 3 recruitments appear in 1986 and 1987 in all models that contribute to high exploitable abundances in 1988 and 1989. Age composition data (Table 1) show similar strong recruit years in 1986 and 1987. Subsequent declines in age 3 recruits are reflected in a declining trend in exploitable abundances. For all models, recruitment appears

Fig. 1. Estimates and bootstrapped 2.5 and 97.5 percentiles (vertical lines) for exploitable abundance of humpback whitefish from catch-age models by year, compared with mark–recapture estimates. ●, base; ○, CV^{-2}; ▲, perception; △, combined. Boxes are the mark–recapture 95% CI.



to be linearly related to exploitable abundance for the years 1989–1992 (Fig. 2 shows this relationship for the perception model).

Catch-age models

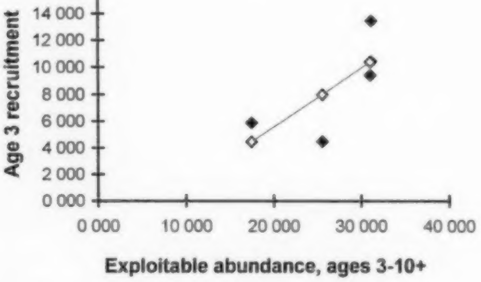
Model fit of the combined and CV^{-2} weighting schemes in the early years of harvest is poor (Fig. 3), possibly evidence of misspecified model dynamics; the θ weighting schemes in these models place the least emphasis on harvest in 1986 and 1987. Sums of residuals are greatest in the combined and CV^{-2} weighting schemes. Lehmann's test showed that the RMSs of the harvest data set for all model pairs were significantly different ($p < 0.01$) except the base–perception pair. Thus, the base and perception models are preferred over the combined and CV^{-2} models with regard to the harvest data set. RMSs in the abundance and age composition data sets do not significantly differ among the models; no model is preferred over another with regard to RMSs in the abundance and age composition data sources.

RMSE in exploitable abundance, exploitation rate, and recruitment is greater for the combined and CV^{-2} models than for the base and perception models (Fig. 4). Thus, the θ weighting scheme in the base and perception models provides parameter estimates with the most precision and least bias.

Forecasting exploitable abundance

The choice of a sustainable exploitation rate is greatly dependent on the recruitment scenario. With constant recruitment, fish abundance tends to remain steady beginning in 1996, but at diminishing levels with increasing exploitation (Fig. 5). With density-independent recruitment, the stock does not remain above the minimum threshold of 10 000 fish over the forecast period at an exploitation of 10% or greater (Fig. 5). The width between the 2.5 and 97.5 percentile ranges increases over time with constant recruitment, demonstrating greater uncertainty in the expected long-run forecast by 1998.

Fig. 2. Relationship between exploitable and prefishery abundance of age 3 recruits lagged 3 years for the perception model (r^2 = 0.494). ◆, Y; ◇, predicted Y).



Results are summarized in a decision table (Table 4), which provides the manager information about the probabilities of being below the threshold for each exploitation strategy under various abundance and recruitment scenarios, as obtained by tabulations of the bootstrap abundance forecasts.

Choosing the best model

Overall model ratings (derived from weighted scores) vary depending on the criterion (see Table 5), and without a technique for synthesizing all aspects involved in selecting the best model, the selection process would be arbitrary. The perception model ranks highest overall (T_m = 0.337), based on rank order among criteria and weights of importance as-

Fig. 3. Residuals for the (*a*) abundance and (*b*) harvest data sets and (*c*) likelihood components for age composition from catch-age models by year. There was no abundance estimate in 1990 and no harvest in 1991. ●, base; ○, CV^{-2}; ▲, perception; △, combined.

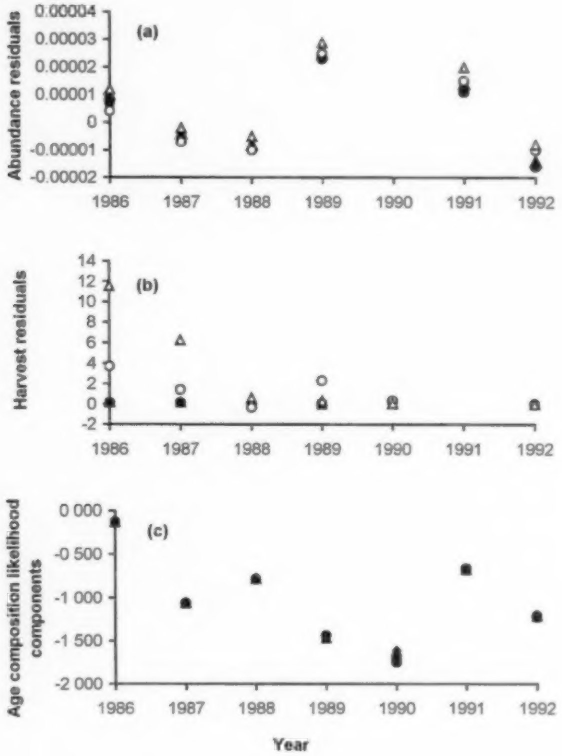
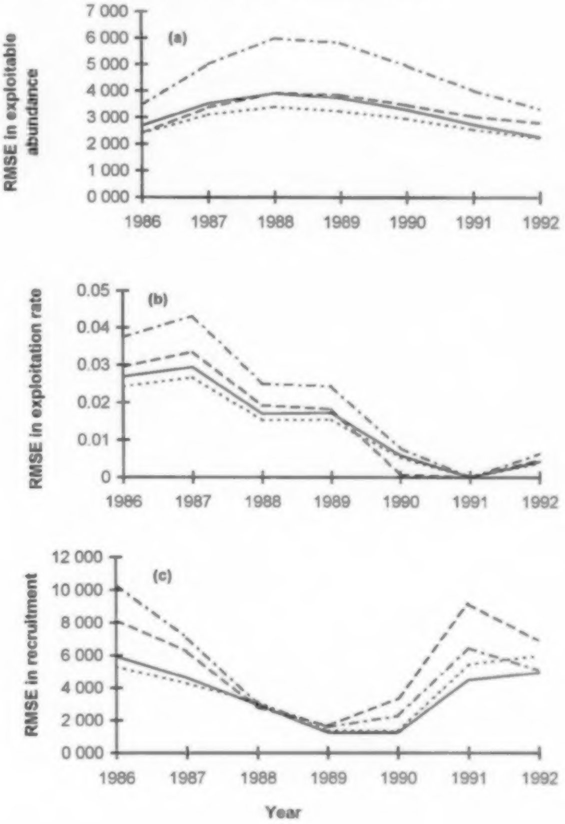


Fig. 4. Root MSE in estimated (*a*) exploitable abundance, (*b*) exploitation rate, and (*c*) recruitment from catch-age models by year. Solid line, perception; short-dashed line, base; long- and short-dashed line, combined; long-dashed line, CV^{-2}.



signed to criteria (Fig. 6). Because plausibility was the same for all models, the superiority of the perception model is due to its excellent fit of the harvest data, relatively low parameter uncertainty, and low forecast risk.

Discussion

Rather than accept uncertain data as equally reliable, or entirely discount these data, this study explores a methodology for explicitly weighting observations according to prior perceptions of accuracy. Our methodology is not a full Bayesian treatment as in McAllister and Ianelli (1997). The Bayesian uses probability theory to interpret the indefiniteness of one's opinions, arguing that point values of belief are actually interval valued. However, surrounding the point estimate of belief with an interval does not necessarily bring greater realism to the model outcome. In situations where an exact value of belief can be numerically expressed with confidence, then a briefer form of the full Bayesian analysis, such as presented in this paper, will bring economy to the assessment method. Explicit weighting to incorporate prior beliefs narrows the field of possibilities and is simple in its execution. Instead of modeling using a probability distribution, we use explicit weights based on perception of data accuracy, which are placed on annual estimates of harvest, age composition, and auxiliary mark–recapture estimates of abundance. Both heuristic and Bayesian methods seek to deal with subjectivity explicitly in minimizing estimation error.

In situations where information has been collected under varying circumstances and is uncertain, and there is an individual (or group) familiar with the assessment program, perception-weighted models may offer advantages over other weighting schemes. One disadvantage of including perceptions of several people in the weighting scheme is that human choice behavior in comparing among data sources or annual estimates is affected by many biases (Hogarth, cited in Poyhonen et al. 1994). In combining individual perceptions, it is assumed that their scales of comparison are consistent (see Keeney and Raiffa 1976). In this study, perceptions were elicited from one fishery manager who was familiar with all aspects of the entire assessment program. A long data series may necessitate the combination of numerical perceptions from several individuals.

Effects of weighting schemes on model results

Weights (θ) attached to annual O components represent uncertainty about the precision and (or) accuracy of the data. Selection of annual weights of influence is based on prior

Fig. 5. Forecasted exploitable abundance for humpback whitefish at two recruitment scenarios (constant, density independent) and three exploitation rates (0, 0.10, and 0.15) for the perception model. The dashed line is the 95% CI.

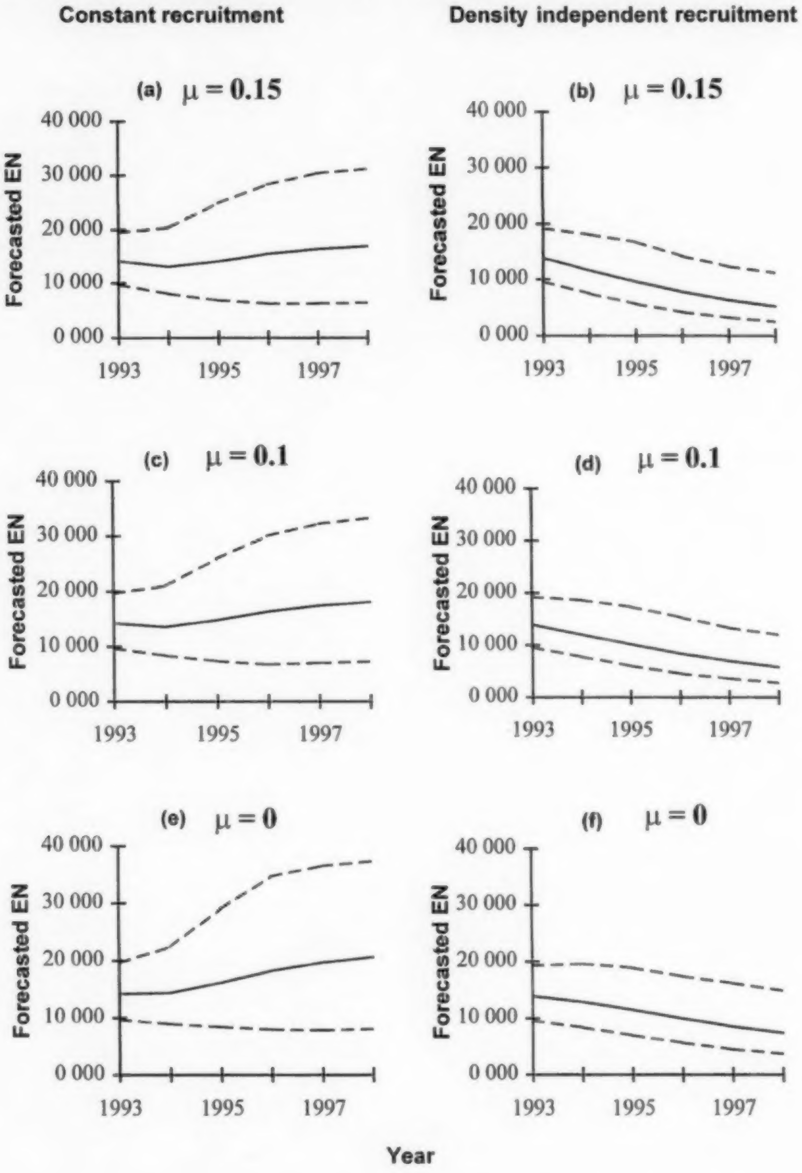
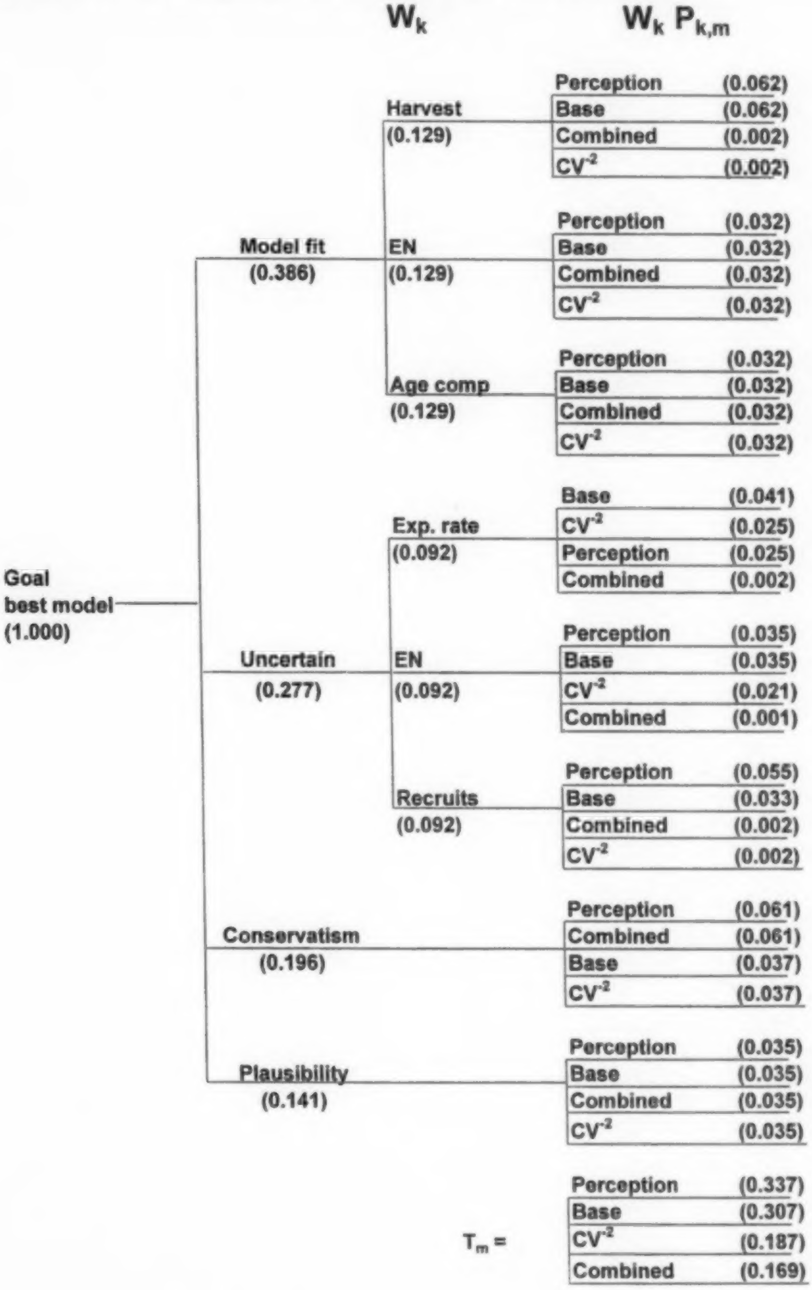


Table 4. Probability that forecasted exploitable abundance during 1993–1998 is below the minimum threshold of 10 000 fish, by assessment model, for two recruitment scenarios and three exploitation strategies.

	Constant recruitment			Density-independent recruitment		
Assessment model	$\mu = 0$	$\mu = 0.10$	$\mu = 0.15$	$\mu = 0$	$\mu = 0.10$	$\mu = 0.15$
Base	0.054	0.092	0.120	0.342	0.455	0.505
CV^{-2}	0.065	0.102	0.117	0.261	0.341	0.389
Perception	0.087	0.132	0.155	0.421	0.544	0.591
Combined	0.125	0.196	0.199	0.429	0.524	0.568

Fig. 6. Hierarchy of criteria and models showing the weight of importance for the criteria (W_k), the weighted proportion of the total score by model ($W_kP_{k,m}$), and the sum of each model's score (T_m) using the analytic hierarchy process.


W_k
W_k P_k,m
Goal
best model
(1.000)
Model fit
(0.386)
Harvest
(0.129)
Perception (0.062)
Base (0.062)
Combined (0.002)
CV^-2 (0.002)
EN
(0.129)
Perception (0.032)
Base (0.032)
Combined (0.032)
CV^-2 (0.032)
Age comp
(0.129)
Perception (0.032)
Base (0.032)
Combined (0.032)
CV^-2 (0.032)
Uncertain
(0.277)
Exp. rate
(0.092)
Base (0.041)
CV^-2 (0.025)
Perception (0.025)
Combined (0.002)
EN
(0.092)
Perception (0.035)
Base (0.035)
CV^-2 (0.021)
Combined (0.001)
Recruits
(0.092)
Perception (0.055)
Base (0.033)
Combined (0.002)
CV^-2 (0.002)
Conservatism
(0.196)
Perception (0.061)
Combined (0.061)
Base (0.037)
CV^-2 (0.037)
Plausibility
(0.141)
Perception (0.035)
Base (0.035)
Combined (0.035)
CV^-2 (0.035)
T_m =
Perception (0.337)
Base (0.307)
CV^-2 (0.187)
Combined (0.169)

information regarding empirical evidence (variance estimates) or personal perceptions. Weights associated with each source of information (λ) reflect attempts to bring these *O*s closer in magnitude for use in the nonlinear minimization procedure. The process for selecting λs is more arbitrary in nature than that for θs. Further research on λ scaling coefficients is needed for robust weighting systems when annual weightings are used.

The RMSs are significantly different among models for the harvest data set. In both the CV^{-2} and combined models, the fit to the observed data in the early years of harvest is poor. The least weight in these models is applied to the har-

Table 5. Scores ($r_{k,m}$) by criterion (k) and model (m) used in finding the weighted overall rating (T_m) of each model.

	Model (m)			
Criterion (k)	Base	CV^{-2}	Perception	Combined
Model fit				
Harvest	5	1/5	5	1/5
Exploitable abundance	5	5	5	5
Age composition	5	5	5	5
Parameter uncertainty				
Exploitation rate	5	3	3	1/5
Exploitable abundance	5	3	5	1/5
Recruits	3	1/5	5	1/5
Conservatism[a] EN($p <$ 10 000), $\mu = 15\%$	3	3	5	5
Plausibility	5	5	5	5

[a] The constant recruitment scenario is presented. Results are similar for the density-independent recruitment scenario.

vest data set in 1986 and 1987 because those years of large harvests were associated with the highest variances. The slight emphasis of the θ weighting scheme in years when harvests were high resulted in possibly misspecified model dynamics for the CV^{-2} and combined models. Incorporating perception resulted in conservative estimates of exploitable abundance under both the constant and density-independent recruitment scenarios because the highest weights of influence are placed on years (1991 and 1992) in which ages 3–5 fish comprise the smallest proportion of the population (0.13 for both years).

Because it is the magnitude of the weights that contributes to how a particular weighting scheme will affect the results, generalizations about the merits of variance or perception weights, or a combination of the two, are premature. Thus, several weighting schemes should be explored in applications. In this application, the perception model produces the most conservative strategy to avoid overfishing humpback whitefish in the Chatanika River. Decisions based on perceptions of data accuracy are always present to some extent in empirical analyses. This study makes these perceptions explicit and quantifies their influence on a given model solution. There are limited alternatives to the manager. Discarding data from flawed studies is inefficient, arguing that there is no signal in the data, only noise. Alternatively, if suspect data are accepted as equal to reliable data, the influence of the "sound" observations is diluted.

Choosing the best model

Because the true state of the population is not generally known, choosing a stock assessment model is not simple. The analytic hierarchy process enabled us to determine the relative importance of four criteria (model fit, parameter uncertainty, conservative forecasts of exploitable abundance, and biological plausibility) that we considered crucial to model selection. It is typical for risk of the harvest strategy to be evaluated and a preference given to a conservative harvest policy (Food and Agriculture Organization of the United Nations 1995). The idea that one should prefer an estimation procedure because it results in a low estimate of abundance or biomass is a concept that is receiving more attention. The advice to managers contained in Table 4 is valuable because it presents the probability of the stock being below threshold, depending on recruitment scenario and harvest strategy. Selecting the stock assessment model with lower biomass estimates, and hence with higher probabilities of being below a fixed abundance level, establishes a precautionary approach to forecasts of exploitable abundance. In a conservative context, fishery management decisions should include considerations of risk to the stock in the setting of harvest policy *and* in the selection of the assessment model.

It is instructive to note that the risk measure is interpreted differently in these two situations. In evaluating harvest policy, within a particular stock assessment model, a policy with a lower probability of dropping below the threshold is more risk averse because the model is considered correct in the evaluation. In evaluating a suite of stock assessment models, a policy with a higher probability of dropping below the threshold is more risk averse because one is accounting for the uncertainty in the model being wrong.

Conservatism is not the only criterion that should go into model selection and harvest policy. In our case, the combined model had the highest conservatism in terms of the probability of future abundance being below the threshold but was the lowest ranked model because of its poor fit to the harvest data and most uncertain parameter estimates compared with other models.

Implications for management

We examined the consequences of several exploitation rates on the humpback whitefish population under a threshold harvest policy by using forecasts of exploitable abundance. A variety of outcomes could result from the current management policy based on the recruitment scenarios examined. From these outcomes, the probability that stock abundance will be below a threshold level of 10 000 fish is presented up to 6 years into the future. Exploitable abundance is sustained above the threshold of 10 000 fish with constant recruitment. Not surprisingly, the probability of abundance falling below the threshold increases with exploitation rate and if density-independent recruitment is present. Thus, a sustainable recreational fishery for humpback whitefish under the current management policy is when periodic strong year-classes occur. Otherwise, the density-independent scenario shows that the possibility of a nonsustainable harvest is high.

One feature of catch-age analysis is that current population abundance estimates can vary substantially as more data become available (Parma 1993). Thus, the strength of catch-age analysis may not be so much in providing current information for management, but rather as a tool for incorporating weights, such as perceptions, into a structured framework to quantify the risks to the population associated with various harvest strategies. Because the catch-age model developed in this study conforms to the error structure of data collected for recreational fish assessment, it is possible that the model can have wide application in recreational fisheries problems.

Acknowledgments

Partial funding for this research was provided through the

Alaska Cooperative Fish and Wildlife Research Unit, University of Alaska Fairbanks, and the Sport Fish Division, Alaska Department of Fish and Game. We thank John H. Clark and James B. Reynolds for encouragement and support. Keith Criddle introduced us to the analytic hierarchy process and provided guidance in decision theory. Linda Brannian and Fritz Funk offered advice regarding the application of a spreadsheet approach to catch-age analysis. We thank Jie Zheng, Fritz Funk, Milo Adkison, Philip Symons, and Martin Dorn for helpful comments on the manuscript.

References

Abraham, B., and Ledolter, J. 1983. Statistical methods for forecasting. John Wiley & Sons, New York.

Badinelli, R.D., and Baker, J.R. 1990. Multiple attribute decision-making with inexact value-function assessment. Decision Sci. **21**: 318–334.

Clark, C.W. 1985. Bioeconomic modeling and fisheries management. John Wiley & Sons, New York.

Criddle, K.R., and Havenner, A.M. 1991. An encompassing approach to modeling fishery dynamics: modeling dynamic nonlinear systems. Nat. Resour. Model. **5**: 55–90.

Deriso, R.B., Quinn, T.J., II, and Neal, P.R. 1985. Catch-age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. **42**: 815–824.

Efron, B., and Tibshirani, R.J. 1993. An introduction to the bootstrap. Chapman and Hall, New York.

Food and Agriculture Organization of the United Nations. 1995. Part 1. Guidelines on the precautionary approach to capture fisheries and species introductions. FAO Fish. Tech. Pap. 350/1.

Francis, R.I.C.C., and Shotton, R. 1997. Risk in fisheries management: a review. Can. J. Fish. Aquat. Sci. **54**: 1699–1715.

Fukushima, M., Quinn, T.J., II, and Smoker, W.W. 1998. Estimation of eggs lost from superimposed pink salmon (*Oncorhynchus gorbuscha*) redds. Can. J. Fish. Aquat. Sci. **55**: 618–625.

Funk, F., Brannian, L.K., and Rowell, K.A. 1992. Age-structured assessment of the Togiak herring stock, 1978–1992, and preliminary forecast of abundance for 1993. Regional Inf. Rep. No. 5J92-11. Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau, Alaska.

Geiger, H., and Koenings, J. 1991. Escapement goals for sockeye salmon with informative prior probabilities based on habitat considerations. Fish. Res. **11**: 239–256.

Hilborn, R., Pikitch, E.K., and McAllister, M.K. 1994. A Bayesian estimation and decision analysis for an age-structured model using biomass survey data. Fish. Res. **19**: 17–30.

Keeney, R.L., and Raiffa, H. 1976. Decisions with multiple objectives. John Wiley & Sons, New York.

Kimura, D.K. 1990. Approaches to age-structured separable sequential population analysis. Can. J. Fish. Aquat. Sci. **47**: 2364–2374.

Martin, J.B. 1979. The use of probability distributions in fisheries decision making. Alaska Sea Grant Program; Alaska fisheries: 200 years and 200 miles of change. Proceedings of the 29th Alaska Science Conference. University of Alaska Fairbanks, Fairbanks, Alaska.

McAllister, M.K., and Ianelli, J. 1997. Bayesian stock assessment using catch-age data and the sampling-importance resampling algorithm. Can. J. Fish. Aquat. Sci. **54**: 284–300.

Merritt, M.F. 1995. Application of decision analysis in the evaluation of recreational fishery management problems. Ph.D. thesis, University of Alaska Fairbanks, Fairbanks, Alaska.

Methot, R.D. 1990. Synthesis model: an adaptable framework for analysis of diverse stock assessment data. *In* Proceedings of the Symposium on Application of Stock Assessment Techniques to Gadids. *Edited by* L.-L. Low. Int. North Pac. Fish. Comm. Bull. **50**: 259–277.

Parma, A.M. 1993. Retrospective catch-at-age analysis of Pacific halibut: implications on assessment of harvesting strategies. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse, D. Eggers, R. Marasco, C. Pautzke, and T. Quinn, II. Alaska Sea Grant College Program AK-SG-93-02. pp. 247–265.

Pearse, P.H., and Walters, C.J. 1992. Harvesting regulation under quota management-systems for ocean fisheries — decision-making in the face of natural variability, weak information, risks and conflicting incentives. Mar. Policy, **16**:167–182.

Poyhonen, M.A., Hämäläinen, R.P., and Salo, A.A. 1994. An experiment on the numerical modeling of verbal ratio statements. Helsinki Univ. Technol. Syst. Anal. Lab. Res. Rep. A50. Otakaari 1M, Espoo, Finland.

Quinn, T.J., II, and Deriso, R.B. 1999. Quantitative fish dynamics. Oxford University Press, New York.

Quinn, T.J., II, Fagen, R., and Zheng, J. 1990. Threshold management policies for exploited populations. Can. J. Fish. Aquat. Sci. **47**: 2016–2019.

Saaty, T.L. 1990. Decision making for leaders. University of Pittsburgh, Pittsburgh, Pa.

Seber, G.A. 1982. The estimation of animal abundance and related parameters. 2nd ed. Charles Griffin and Company, Limited, London, U.K.

Srinivasan, V., and Shocker, A.D. 1973. Estimating the weights for multiple attributes in a composite criterion using pairwise judgments. Psychometrika, **38**: 473–493.

Steel, R.G., and Torrie, J.H. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York.

Tait, J. 1988. The role of values in quantitative decision-making. J. Oper. Res. Soc. **39**: 669–674.

Thompson, S.K. 1992. Sampling. John Wiley & Sons, New York.

Williams, E., and Quinn, T.J., II. 1998. A parametric bootstrap of catch-age composition using the Dirichlet distribution. *In* Proceedings of the International Symposium on Fishery Stock Assessment Models. *Edited by* F. Funk, T.J. Quinn, II, J. Heifetz, J.N. Ianelli, J.E. Powers, J.F. Schweigert, P.J. Sullivan, and C.-I. Zhang. Alaska Sea Grant College Program AK-SG-98-01. pp. 371–384.

Zheng, J., Quinn, T.J., II, and Kruse, G.H. 1993. Comparison and evaluation of threshold estimation methods for exploited fish populations. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse, D. Eggers, R. Marasco, C. Pautzke, and T. Quinn, II. Alaska Sea Grant College Program AK-SG-93-02. pp. 267–289.

Geomorphology, hyporheic exchange, and selection of spawning habitat by bull trout (*Salvelinus confluentus*)

Colden V. Baxter and F. Richard Hauer

Abstract: The distribution and abundance of bull trout (*Salvelinus confluentus*) spawning were affected by geomorphology and hyporheic groundwater – stream water exchange across multiple spatial scales in streams of the Swan River basin, northwestern Montana. Among spawning tributary streams, the abundance of bull trout redds increased with increased area of alluvial valley segments that were longitudinally confined by geomorphic knickpoints. Among all valley segment types, bull trout redds were primarily found in these bounded alluvial valley segments, which possessed complex patterns of hyporheic exchange and extensive upwelling zones. Bull trout used stream reaches for spawning that were strongly influenced by upwelling. However, within these selected reaches, bull trout redds were primarily located in transitional bedforms that possessed strong localized downwelling and high intragravel flow rates. The changing relationship of spawning habitat selection, in which bull trout selected upwelling zones at one spatial scale and downwelling zones at another spatial scale, emphasizes the importance of considering multiple spatial scales within a hierarchical geomorphic context when considering the ecology of this species or plans for bull trout conservation and restoration.

Résumé : La distribution et l'abondance de l'omble à tête plate (*Salvelinus confluentus*) en phase de reproduction étaient affectées par la géomorphologie et par l'échange d'eau entre la nappe sous-terraine hyporhéique et les cours d'eau à des échelles spatiales multiples dans les cours d'eau du bassin de la Swan, nord-ouest du Montana. Parmi les affluents de fraye, l'abondance des nids d'omble à tête plate augmentait avec la superficie des portions de vallées alluviales qui étaient longitudinalement confinées par des ruptures de pente. Parmi tous les types de portions de vallée, les nids d'omble à tête plate se retrouvaient principalement dans ces portions confinées, qui présentaient des régimes complexes d'échange hyporhéique et de vastes zones de remontée d'eau. L'omble à tête plate recherchait pour la fraye des tronçons fortement influencés par les remontées d'eau. Toutefois, à l'intérieur de ces tronçons sélectionnés, les nids se retrouvaient principalement dans des types de lit de transition qui présentaient une forte remontée d'eau localisée et des taux élevés d'écoulement à l'intérieur du gravier. La relation changeante régissant le mode de sélection de l'habitat de fraye – l'omble de fontaine choisissait les zones de remontée d'eau à une échelle spatiale et les zones d'enfoncement des eaux à une autre échelle spatiale – fait ressortir qu'il est important de considérer des échelles spatiales multiples à l'intérieur d'un contexte géomorphologique hiérarchique lorsqu'on étudie l'écologie de cette espèce ou qu'on fait des plans pour sa conservation et son rétablissement.

[Traduit par la Rédaction]

Introduction

Stream ecologists have long recognized the interactive relationship between a stream and its valley (Hynes 1975). Patterns and processes of stream ecosystems are closely tied to the surrounding terrestrial environment (Junk et al. 1989; Gregory et al. 1991), longitudinal gradients (Vannote et al. 1980; Minshall et al. 1985), and groundwater – stream water exchange (Grimm and Fisher 1984; Stanford and Ward 1993). Because of these interactions, it is essential that stream ecosystems be examined holistically within the context of their landscapes (Frissell et al. 1986; Swanson et al. 1988).

The flow of water over land shapes the geomorphology of stream systems. Geomorphic change, combined with the interactive response of the stream environment, results in alternating erosion and deposition. This dynamic process of cut and fill creates spatial heterogeneity in physical habitat for stream organisms. Stream habitat can be classified hierarchically into drainages, valley segments, stream reaches, and channel bedform units (pool–riffle) (Frissell et al. 1986). Each level of the stream habitat hierarchy corresponds to a distinct spatial scale, from kilometres to metres to centimetres, etc.

At the drainage spatial scale, alluvial river systems of the northern Rocky Mountains are characterized by confined and unconfined valley segments occurring in alternating series along the stream gradient. In unconfined alluvial segments, streams flow across deposits of gravel and cobble associated with alluvial floodplains (Church 1992). These reaches commonly have a vertical dimension of groundwater – surface water interaction extending tens of metres into the alluvium

Received June 29, 1999. Accepted February 29, 2000.
J15222

C.V. Baxter and F.R. Hauer. Flathead Lake Biological Station, University of Montana, 311 Bio Station Lane, Polson, MT 59860-9659, U.S.A.

[1] Author to whom all correspondence should be sent at the following address: Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, U.S.A. e-mail: baxterco@ucs.orst.edu

(Stanford and Ward 1988, 1993). The size and arrangement of particles contained in vertical and lateral profiles of an alluvial segment vary with geomorphic surface. Differences in substrate porosity lead to the formation of preferential hyporheic flow pathways in longitudinal, lateral, and vertical dimensions. In contrast, confined valley segments are generally characterized by narrow valley walls, near-surface bedrock, absence of a floodplain, and relatively high stream gradient (Montgomery et al. 1996).

The response of stream organisms to habitat is scale dependent. Habitat-use patterns for any organism in an ecosystem can be related to many environmental factors. The variation of a habitat factor may be perceived by an organism as information at certain scales, while at another scale, it may be perceived as noise (Dutilleul and Legendre 1993). Consequently, understanding the distribution and abundance of species relative to habitat factors may require a multiscale approach (Wiens 1989). Relationships between fish and their habitat have been primarily examined at fine spatial scales (Bayley and Li 1992). However, there is increasing recognition of the importance of a multiscale approach (e.g., Poizat and Pont 1996; Torgersen et al. 1999) and inclusion of critical landscape features such as zones of process interaction (e.g., land–water ecotones) (Schlosser 1995).

The hyporheic zone is an ecotone that links ground and surface water processes in streams (Brunke and Gonser 1997). The boundaries of groundwater – stream water interactions are spatially and temporally dynamic because of fluctuating hydrographic regimes. Although hyporheic exchange has been hypothesized to occur at multiple spatial scales (e.g., drainage, valley segment, reach, and channel unit scales; Stanford and Ward 1993), few efforts have been made to quantify hyporheic exchange extensively within a spatial hierarchy (Boulton et al. 1998). Most investigations of groundwater – surface water interaction in streams have focused at the channel bedform scale (i.e., pool–riffle, which includes local, shallow exchange). Hyporheic exchange at this spatial scale is known to vary with discharge, gradient, bedform, sediment permeability, and water surface slope (Vaux 1968; Thibodeaux and Boyle 1987; Harvey and Bencala 1993). Few studies of hyporheic exchange have been conducted at anything larger than the reach scale; however, theoretical models predicting how the hyporheic zone changes within a drainage have recently been proposed (e.g., Stanford and Ward 1993; White 1993).

For decades, fisheries research involving groundwater – stream water exchange has primarily focused on the importance of intragravel flow to the incubation of fish embryos (e.g., Terhune 1958; Curry and Noakes 1995). Upwelling water often differs from surface water in numerous ways (e.g., dissolved oxygen, nutrients), but perhaps none more important than temperature regime. Upwelling waters in temperate climates are generally cooler in summer and warmer in winter and may provide thermal refugia for stenothermic fish species (e.g., Gibson 1966; Nielson et al. 1994).

The bull trout (*Salvelinus confluentus*) is a native char in the Pacific Northwest whose populations are fragmented and declining throughout their range due to overharvest, displacement by exotic species, and habitat degradation (Rieman and McIntyre 1993). In 1997 the U.S. Fish and Wildlife Service recommended the bull trout for listing under the U.S. Endangered Species Act. The Swan basin in northwestern Montana (Fig. 1) has received attention as a refuge where many bull trout remain (Rieman and McIntyre 1993; Baxter et al. 1999). Consequently, this basin provides an opportunity for developing a better understanding of bull trout in a system where sufficient numbers exist for effective study.

Bull trout display migratory life histories that are associated with large lake and river systems. Migratory bull trout in the Swan basin spawn in second- to fourth-order streams from August through October. Embryos and alevins overwinter in spawning gravel for more than 200 days prior to fry emergence. Juveniles remain in the natal streams for 1–3 years prior to migrating to Swan Lake where they spend 2–4 years growing before sexual maturity (Fraley and Shepard 1989). Factors such as temperature, channel stability, and cover are known to be important spawning habitat characters (for a review, see Rieman and McIntyre 1993). However, researchers have long recognized that many stream segments of ostensibly suitable habitat (i.e., substrata, gradient, and cover) were never used by spawning bull trout. For example, over 75% of the spawning in the Swan basin occurs in less than 10% of the available stream length (Leathe and Enk 1985). Although studies have noted that stream habitats selected by bull trout for spawning are often influenced by groundwater (Graham et al. 1981; Weaver and White 1985), a quantitative spatial assessment of this habitat factor and its importance to bull trout has not yet been conducted. Furthermore, no studies have focused on a multiscale spatial assessment of hyporheic exchange and spawning habitat use patterns.

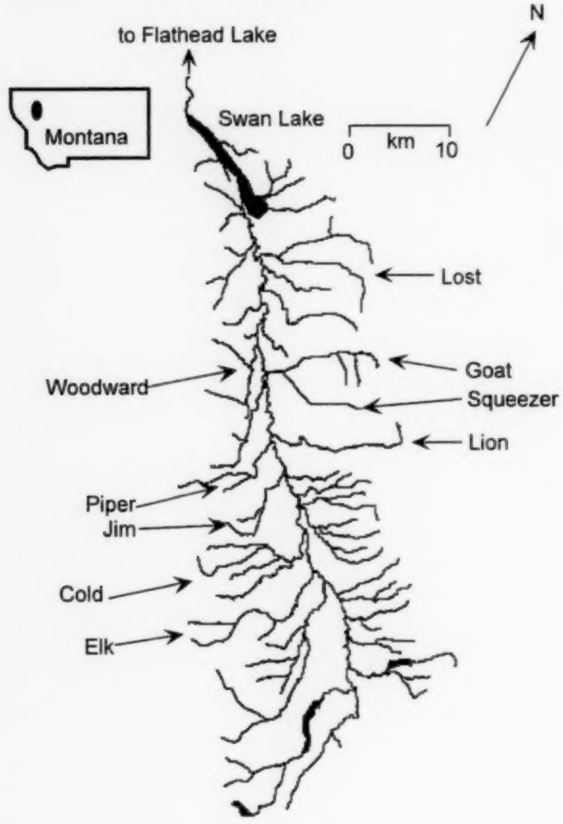
The objective of this study was to examine the relationships between geomorphology, groundwater – stream water exchange patterns, and the distribution and abundance of bull trout spawning sites. General observations of geomorphic structure at the landscape scale, recent study of hyporheic exchange in large, coarse-substratum alluvial floodplains (Stanford and Ward 1993), and patterns of bull trout spawning (e.g., Weaver and White 1985) led us to hypothesize that these biophysical variables were related. Our specific research objectives were to (*i*) quantify the process of hyporheic exchange at multiple spatial scales, (*ii*) identify geomorphic features associated with variation of hyporheic exchange at each spatial scale, and (*iii*) evaluate the influence of hyporheic exchange within the context of a geomorphic hierarchy (i.e., drainage, valley segment, reach, and bedform scales) on the distribution of bull trout spawning.

Materials and methods

Study area

The Swan basin (2070 km^2) is a densely forested, north–south trending, glaciated basin between the Swan and Mission fault-block mountain ranges in northwestern Montana. From peak elevations over 2500 m that are in excess of 1500 m above the valley floor, waters drain through tributary canyons carved in Precambrian metamorphic rock. Tributary streams reach their confluence with the sinuous Swan River as it flows across morainal and alluvial deposits of the broad Swan Valley north into Swan Lake (Fig. 1). The current geomorphic character of the Swan River and its tributaries was established during two major Pleistocene glacial advances, which led to a period of fluvial transport, mass wasting, and alluvial thickening by in-filling from glacial outwash (Kleinkopf et al. 1972). Streams in the Swan basin traverse valley

Fig. 1. Map of the Swan River basin (latitude 47°35′, longitude 113°45′). The nine primary bull trout spawning tributary streams are identified by name. The inset indicates the location of the Swan River in northwestern Montana.



floors dominated by mixed-conifer forest (e.g., *Abies*, *Pinus*, *Thuja*, *Pseudotsuga*, *Picea*, *Larix*), with deciduous trees and shrubs (e.g., *Betula*, *Populus*, *Salix*) concentrated along riparian areas and floodplains.

Our study area included nine third- and fourth-order (Strahler 1964) tributary streams, which constitute the principal bull trout spawning tributaries of the Swan River (Leathe and Enk 1985). The nine tributary drainages ranged in size from 32.9 km^2 (Piper Creek) to 81.8 km^2 (Lion Creek). These streams are typical of the northern Rocky Mountains, possessing a snowmelt-dominated hydrograph with peak flows in late May or June as much as three orders of magnitude greater than base flows. Streams are further characterized by large cobble substratum and waters that are generally very low in dissolved ions, nutrients, and suspended particulates.

Characterization of geomorphic features

At the drainage scale, we used standard U.S. Geological Survey topographic 7.5′ quadrangle maps (1 : 24 000 scale) and structural geological maps (1 : 250 000 scale) to identify and measure 28 geomorphic variables for each of the nine study drainages (Fig. 1; Table 1). We also used aerial photographs and field verification to aid in discrimination of specific features. In addition to traditional landscape-scale geomorphic metrics (Strahler 1964), we classified valley segments into three categories: (*i*) bounded alluvial valley segments, (*ii*) unbounded alluvial valley segments, and (*iii*) confined valley segments.

Bounded alluvial valley segments were characterized by unconfined stream sections at least 500 m in length and greater than 50 m in valley bottom width with a downstream geomorphic knickpoint. Generally, knickpoints were distinguished by steepening in channel slope, constriction in valley width, and a geologic formation near the surface, such as bedrock or terminal moraine deposits. By including measures of valley bottom width and the number of knickpoints, we were able to estimate the number, length, and area of bounded alluvial valley segments (Fig. 2). The area of bounded alluvial segments was examined because it helped describe the overall valley shape that we hypothesized was important to the structuring of hyporheic flow patterns (Fig. 2). Unbounded alluvial valley segments were similar to bounded alluvial valley segments but were always the furthest downstream segment of a tributary where the stream emerged onto the Swan Valley floor and flowed to the Swan River without encountering another knickpoint. We identified confined valley segments by their narrow valley bottoms (typically <50 m) and steep gradients (usually <0.05 m/m). Confined valley segments often were associated with canyons and near-surface bedrock with only a patchy, thin (<2 m) layer of cobble substratum.

Characterization of hyporheic exchange

We quantified hyporheic exchange by measuring vertical hydraulic gradient (VHG) and streambed hydraulic conductivity. We installed piezometers in the stream substratum and calculated VHG from the equation

$$\mathrm{VHG} = \mathrm{d}h/\mathrm{d}l$$

where dh is the difference in head between the water surface in the piezometer and the level of the stream surface (the hydraulic head differential, centimetres), and dl is the depth from the substratum surface to the first opening in the piezometer sidewall (the elevation head differential, centimetres). Thus, VHG is a unitless measure that is positive under upwelling conditions and negative under downwelling conditions.

Hydraulic conductivities (K) were estimated using falling head tests (Lee and Cherry 1978), which measure the rate of water-level equilibration between the piezometer and the surrounding groundwater. For some piezometers, we were able to estimate K using the Hvorslev (1951) equation:

$$K = \frac{(\pi)(D)}{(11)(T_0)}$$

where D is the inside diameter of the piezometer and T_0 is the basic time lag, a value derived from the construction of an equilibration curve (for summary of calculation, see Cedergren 1989). However, among many of the piezometers, equilibration took place so quickly that it was impossible to use the conventional falling head test to create a curve and acquire an accurate estimate of K. Thus, we derived an alternative equation:

$$K = \frac{(0.2501)(D)}{(d_t)}\left|\ln\frac{h_0}{h}\right|$$

where D is the inside diameter of the piezometer and d_t is the time it takes for the head level to drop from h_0 to h. In the situations where we were able to estimate K using the Hvorslev method as well as our modified equation, there was no appreciable difference between the two estimates. Therefore, for consistency, we used only the values obtained by the latter approach for all our analyses. Following the estimation of K, we were able to calculate the vertical component of flow using Darcy's equation for specific discharge (v, centimetres per second):

Table 1. Drainage-scale geomorphic variables that were examined as a part of this study.

Morphometric measure	Slope class	BAVS[a] related
Drainage area	% drainage <1% slope	Maximum valley bottom width
Stream order	% drainage 1–2% slope	Mean valley bottom width
Total stream length	% drainage 2–7% slope	Variation in valley bottom width
Accessible stream length	% drainage 7–20% slope	Number of gradient steps >10%
Drainage density	% drainage 20–40% slope	Number of BAVS
Drainage length	% drainage 40–55% slope	Total area of BAVS
Average stream gradient	% drainage >55% slope	Length of accessible BAVS
Gradient of accessible stream length	% drainage >55% slope	Area of accessible BAVS
Relief ratio		
Form ratio		
Elongation ratio		
Mean bedrock dip angle		

Note: Accessibility refers to whether migratory bull trout can navigate the stream.

[a] Bounded alluvial valley segment.

$$v = -K \times \mathrm{d}h/\mathrm{d}l$$

where dh and dl are as above for the calculation of VHG (Freeze and Cherry 1979).

We quantified hyporheic exchange at the drainage scale in four of the nine principal bull trout spawning streams: Elk, Lion, Cold, and Lost creeks (see Fig. 1). We installed more than 500 piezometers in alluvial valley segments of these drainages. We did not sample confined segments for VHG because generally, it was impossible to install piezometers in the large, boulder-dominated bed material typical of the confined segments, and frequently confined segments were characterized by near-surface bedrock.

Alluvial valley segments within each of the four tributary drainages were divided into upper, middle, and lower regions, and a reach of approximately 200 m length was sampled in each. Piezometers were spaced at 10- to 15-m intervals, and a minimum of 15 piezometers were installed in each reach. At this scale of sampling, we measured only VHG for each piezometer (no estimate of K). To examine the relative potential for upwelling at each piezometer site, we transformed these VHG data by changing all negative values to zero, while all positive values remained the same. For the sake of simplicity, we refer to these values as groundwater input, although they are actually only a measure of input potential.

We examined hyporheic exchange and streambed geomorphology at the bedform (pool–riffle) spatial scale through detailed analysis of two spawning reaches in Cold and Lion creeks. Following installation of an extensive network of more than 50 piezometers in 100 m of stream length, we measured VHG and K. We then used a Lietz TC600 Total Station to survey channel bedform, topographic complexity of the surrounding floodplain, and piezometer and bull trout redd locations by acquiring 600–700 individual point readings. We then constructed detailed planimetric maps of floodplain and channel morphometry, bull trout redds, and piezometer locations using SURFER$^{\mathrm{TM}}$ mapping software. We also constructed topographic contour maps and interpolated isopleths of VHG and intragravel v. Channel morphometry maps were used to separate the streambed into three categories based on longitudinal bedslope character: (*i*) concave, (*ii*) convex, and (*iii*) transitional. Concave patches typically were associated with riffles and the head of pools and convex areas with pool tailouts, and transitional patches were located at the downstream edge of pool tailouts that merge into the head of a riffle. Because we did not want to disturb bull trout eggs, piezometers were not installed directly into redds.

Bull trout redds and stream temperature

Data on the distribution and abundance of bull trout redds were collected in two ways. In all nine principal spawning streams of the Swan basin, we obtained redd data from surveys conducted by the Montana Department of Fish, Wildlife and Parks in autumn 1995. The precision of these data was sufficient for drainage and valley segment scale analyses and allowed us to position bull trout redd locations on topographic 7.5′ quadrangle maps that had geomorphic variables delineated for landscape characterization. We also conducted our own redd surveys on Elk, Lion, Cold, and Lost creeks in order to map locations of redds with the greater precision required for reach and channel unit scale analyses.

We installed Onset$^{\mathrm{TM}}$ data loggers to monitor water temperature within the four primary study drainages. We placed temperature loggers in stream reaches that our VHG data indicated to be reaches of groundwater discharge and in reaches known to be either VHG neutral or VHG negative. We also conducted several winter surveys of each creek to estimate the percent ice cover and document the occurrence or absence of anchor ice in proximity to positive or negative VHG readings from our piezometers.

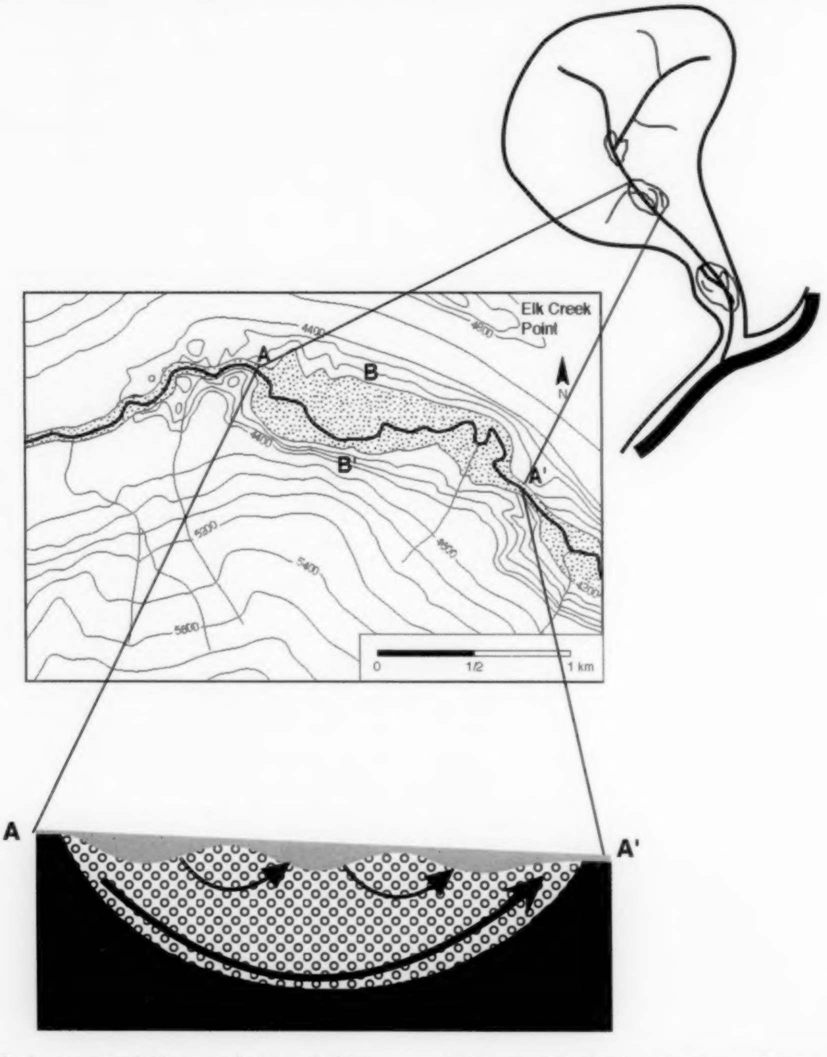
Data analysis

We utilized an empirical approach to assess the distribution of bull trout spawning with respect to geomorphology and hyporheic exchange. At each spatial scale (i.e., drainage, valley segment, reach, and channel unit), we expected that the distribution of bull trout redds would take one of two theoretical forms: (*i*) a uniform, even distribution or (*ii*) a discontinuous or patchy distribution. Our working hypothesis was that bull trout redds would be patchily distributed, with specific habitat preference regarding geomorphic and hyporheic exchange variables occurring at each spatial scale. The null hypothesis was that the distribution of bull trout redds is independent of geomorphic or hyporheic exchange variables.

We examined all combinations of dependent and independent variables. At the drainage scale, two correlation matrices were constructed, one that included geomorphic features and bull trout redd numbers for the nine principal bull trout spawning drainages and another that included only the four streams that were sampled for VHG. Similarly, at valley segment and reach scales, one matrix was constructed for all segments and reaches in the nine basins and another for those in which we collected VHG data. These matrices allowed us to examine individual associations between bull trout spawning and landscape-scale geomorphic variables as well as groundwater – surface water exchange variables. They also allowed us to assess covariation among variables. Where we detected significant correlations, we applied linear regression to assess the relationship between dependent and independent variables.

We used nonparametric χ^2 analysis to determine differences between expected and observed patterns of bull trout redd distribu-

Fig. 2. One of the bounded alluvial valley segments (BAVS) in the Elk Creek drainage (latitude 47°30′, longitude 113°48′). The dimensions of the BAVS were measured directly from U.S. Geological Survey topographic 7.5′ quadrangle maps (1 : 24 000 scale) (*A–A′* = length, *B–B′* = maximum valley bottom width). The cross-sectional diagram (*A–A′*) illustrates how reach-scale (large arrow) and bedform-scale (small arrows) hyporheic exchange typically occurs within a BAVS. The stippling denotes the alluvial valley fill.



tion. We also applied nearest-neighbor analysis and Mann–Whitney *U* tests to assess spatial association between bull trout redds and VHGs. When χ^2 tests resulted in significant differences between used and available habitat, we used Jacobs' *D*, a modification of Ivlev's electivity index, as a nonstatistical indication of where differences between use and availability of habitat types occurred (Jacobs 1974). We considered statistical test results to be significant at $\alpha = 0.05$.

Results

Drainage scale

Among the four tributary streams sampled for VHG, the groundwater input, measured as the mean of positive VHG readings and as maximum positive VHG reading, was positively correlated with the area of bounded alluvial valley segments ($P < 0.05$ and $P < 0.01$, respectively). The mean of all VHG values, positive and negative, was significantly associated with only one geomorphic variable, the number of bounded alluvial valley segments ($P < 0.05$) per stream. Drainages with large and frequent bounded alluvial valley segments possessed more stream habitat that was thermally moderated by groundwater discharge than drainages dominated by confined stream segments and unbounded alluvial floodplain segments.

Among the nine principle spawning streams, the abundance of bull trout redds per tributary drainage was not associated with any of the traditional geomorphic metrics, such as drainage area or accessible stream length (Table 1). The distribution and abundance of redds were, however, posi-

Fig. 3. Number of bull trout redds versus the proportion of the total drainage area that was bounded alluvial valley segment (BAVS) for the nine tributary spawning streams in the Swan River basin.

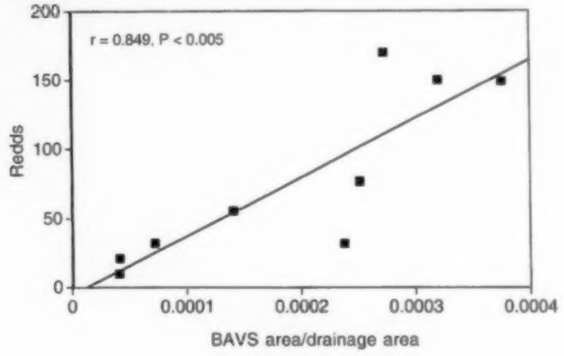
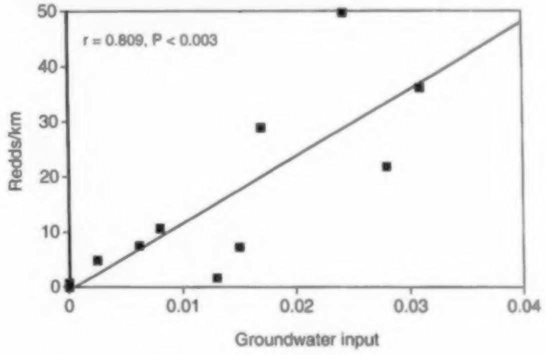


Fig. 4. Number of bull trout redds per stream kilometre in each of the alluvial valley segments sampled for VHG versus the relative strength of groundwater input (mean of transformed VHG readings, unitless) for each valley segment.



tively correlated with geomorphic measures of bounded alluvial valley segments. In particular, the number of redds was positively correlated with bounded alluvial valley area ($P < 0.005$) as well with as the proportion of the drainage area that was bounded alluvial valley ($P < 0.005$) (Fig. 3). In addition, the number of redds was associated with the total area of all bounded alluvial valley segments including those above fish barriers ($P < 0.05$) and with the proportion of the accessible stream length found within bounded alluvial valley segments ($P < 0.05$). Among the four streams sampled for VHG, we found that the relative strength of groundwater input and maximum positive VHG were positively associated with redd counts ($P < 0.05$ and $P < 0.01$, respectively).

Valley segment scale

Within the four streams sampled for VHG, alluvial valley segment types differed significantly in hyporheic exchange depending on whether they were bounded or unbounded by a downstream knickpoint (Mann–Whitney *U*, $P < 0.010$). All bounded alluvial valley segments possessed large and significant upwelling zones, while three of the four unbounded alluvial valley segments were exclusively losing or neutral and the fourth showed only weak zones of positive VHG in isolated sites. We also observed a close association between bounded alluvial valley segment area and the relative strength of groundwater input to a segment ($P < 0.01$). Likewise, bounded alluvial valley segments possessed more stream length that was thermally moderated by groundwater discharge than either unbounded alluvial valley segments or confined segments. They also had the greatest amount of ice-free habitat in the winter.

Among all valley segments in the nine principal spawning streams, bull trout redds were positively associated with bounded alluvial valley segments ($\chi^2 = 917$, $P < 0.0001$). Of the redds observed, 88.5% were located in bounded alluvial valley segments, whereas 8% were found in unbounded alluvial floodplain segments and only 3.5% were observed in confined valley segments. Of the available stream length, 38% was located in bounded alluvial valley segments, 56% in unbounded alluvial valley segments, and 5% in confined segments. The availability of bounded alluvial valley segments relative to their usage suggested a positive selection (Jacobs' $D = 0.450$). Additionally, among the valley segments of the four VHG-sampled tributaries, the relative strength of groundwater input in a segment was positively associated with both the number of redds ($P < 0.05$) and the redd density ($P < 0.005$) (Fig. 4). When only bounded alluvial valley segments were considered, the number of redds was weakly correlated with groundwater input ($P < 0.1$).

Reach scale

Within bounded alluvial valley segments, the geomorphic context of a reach along the longitudinal gradient was often indicative of whether it was a gaining, losing, or neutral reach. Reaches at the upstream end of a bounded alluvial valley segment were usually downwelling, those in the middle were often neutral, and those at the downstream end, just upstream of the bounding knickpoint or valley constriction, were always upwelling. The mean VHG per reach was negatively associated with the mean valley bottom width at each reach ($P < 0.005$). Reaches in the middle of bounded alluvial valley segments had very wide valley bottom widths and were typically neutral or slightly negative in VHG. In addition, several reaches located in the unbounded alluvial valley segments of the four streams had wide valley bottom widths and were strongly downwelling. The thermal regimes of gaining reaches were significantly moderated by the groundwater effect (Fig. 5). Gaining reaches possessed the least ice cover even when the reaches were exposed to extremely cold winter air temperatures. These moderating effects dissipated with increasing downstream distance from the upwelling zone.

Reaches that were gaining water (mean VHG = 0.0169) had the highest number of observed bull trout redds ($n = 94$). Among the reaches sampled, there were no significant differences in redd distribution associated with geomorphic variables (e.g., reach gradient, valley bottom width) other than the proximity to a downstream knickpoint. The number of bull trout redds per reach was positively correlated ($P < 0.001$) with the relative strength of groundwater input per reach (Fig. 6). Furthermore, since the scatter of points on this graph was somewhat clustered, we broke the reaches into categories of low (<0.03) and high (≥0.03) groundwater input and tested the observed versus random distribution of redds among reaches. We found the difference to be highly

Fig. 5. Thermal profiles for two winter weeks from a gaining (solid line) and a losing (broken line) reach of Lion Creek clearly show the moderating effects (both seasonal and diel) of groundwater. The supercooled temperature of the losing reach at the end of the profile corresponded to anchor ice formation. Twenty-three bull trout redds were counted in the gaining reach, while none were found in the losing reach.

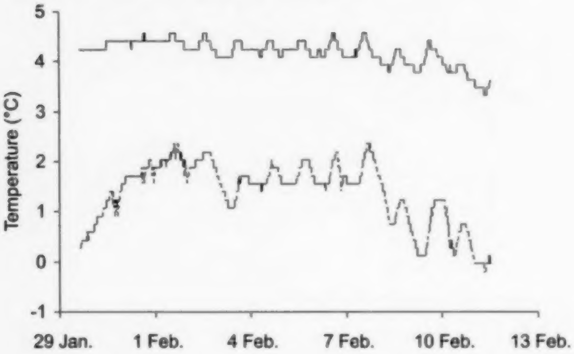
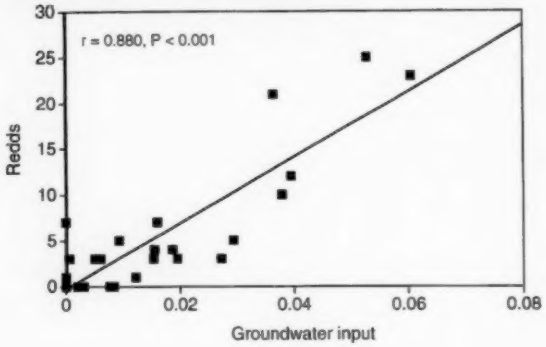


Fig. 6. Number of bull trout redds observed in each of the reaches sampled for VHG versus the groundwater input (mean of transformed VHG readings) for each reach.



significant (χ^2 = 215, P < 0.0001), and reaches with strongly positive groundwater input were selected for spawning based on their availability (Jacobs' D = 0.795).

Pool–riffle scale

Within reaches in Lion and Cold creeks, we observed an association between bedform morphology, hyporheic exchange, and redd distribution. These reaches possessed significant sources of groundwater discharge, had moderated thermal regimes, and were free of ice throughout the winter. However, VHG varied significantly with local bedform character (Fig. 7). Convex and transitional bedforms were significantly different in VHG from concave bedforms (Mann–Whitney U = 64, P < 0.0001), in that areas of the streambed that were transitional or convex possessed negative VHG with means of –0.099 and –0.042, respectively, while concave streambed displayed upwelling VHG (mean = 0.038). In each reach, we observed several instances in which large wood contributed to local variation in bed topography and heterogeneity in the magnitude and direction of VHG. For logs that were positioned perpendicular to the current and that created a pool, shallow downwelling occurred above the logs and upwelling occurred in the pools below the logs. Estimated K ranged from 2.32×10^{-6} cm/s to a maximum of 3.37×10^{-1} cm/s, a difference of more than five orders of magnitude. Falling head test data indicated that transitional bedforms possessed significantly higher intragravel v than either convex or concave bedforms (Mann–Whitney U, P < 0.0001).

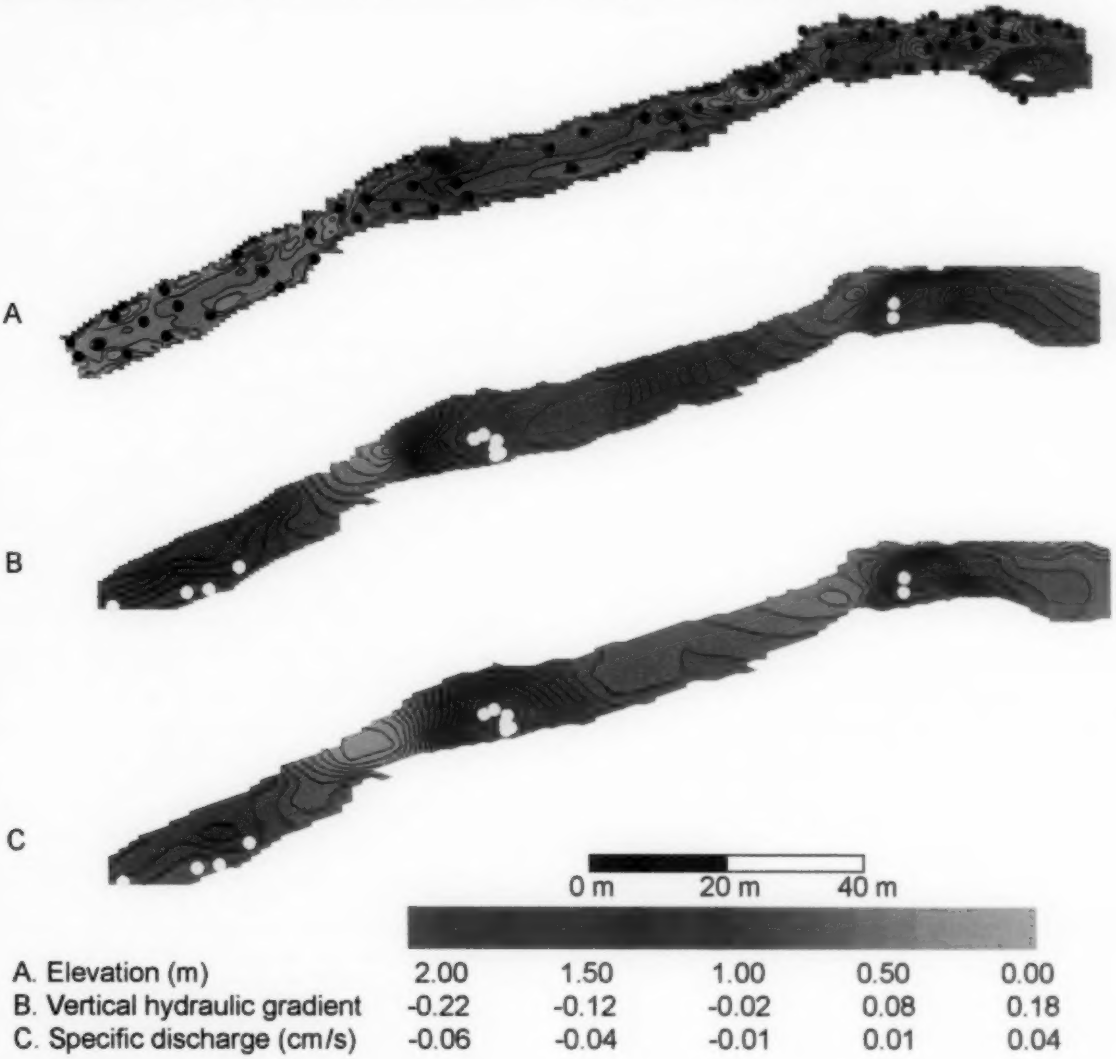
Bull trout redds were located most frequently in transitional as opposed to convex or concave bedform classes (χ^2 = 54, P < 0.0001) (Fig. 7). Of the 15 redds found in the Lion Creek and Cold Creek reaches, only one of these was located in a concave bedform unit. There were two transitional units where no redds were constructed; however, the availability of transitional areas relative to their usage was suggestive of positive selection (Jacobs' D = 0.895). Examination of individual redds revealed that spawning sites were strongly associated with piezometers having a negative VHG to the second nearest neighbor (Mann–Whitney U = 32, P < 0.001). The median distance from redds to the nearest downwelling piezometer was only 2.1 m, while the median distance to the nearest upwelling piezometer was 14.6 m. Furthermore, after accounting for the relative availability of downwelling versus upwelling habitat within these reaches, we found a significant difference between the expected and the observed distributions of redds (χ^2 = 8.99, P < 0.005), suggesting positive selection of transitional bedforms that are also downwelling areas (Jacobs' D = 0.751) (Fig. 7).

The distribution of redds was also associated with the magnitude of intragravel v. Redds were located in areas with the highest flow (indicated by piezometric falling head tests), while those with the weakest flow were never used (Fig. 7). We separated the estimates of v (i.e., v using Darcy's equation) in the two mapped reaches into three categories of low (<0.001 cm/s), medium (0.001–0.01 cm/s), and high (>0.01 cm/s). After accounting for the relative availability of habitat in each intragravel flow category, we found a significant difference between the expected and the observed distributions of redds (χ^2 = 17.98, P < 0.0001), suggesting positive selection of areas with high intragravel v (Jacobs' D = 0.727).

Discussion

The results of this study describe how groundwater – stream water exchange is structured by geomorphic features at drainage, valley segment, stream reach, and channel unit scales. Our findings also show that the selection of spawning sites by bull trout is strongly associated with hyporheic exchange at all of these scales. The mechanisms governing bull trout spawning habitat selection may vary from one scale to another within the hierarchy. Furthermore, although we are unsure of the scale to which homing occurs in this species (Rieman and McIntyre 1993), there is a transition in process that occurs between homing and redd site selection (Northcote 1978). Spawning habitat selection is a complex function of the results of differential survival by embryos and rearing juvenile fish, along with fish sensory capabilities. Although our study does not explicitly determine causal mechanisms, we illustrate how hyporheic exchange may control a variety

Fig. 7. Three maps of a spawning reach on Lion Creek showing (A) channel bed topography and piezometer locations, (B) isopleths of VHG grading from positive VHG (blue) to negative VHG (red), and (C) isopleths of intragravel v grading from high (red) to low (blue) rates. Dots indicate piezometer locations in map A and bull trout redds in maps B and C. The direction of flow is from right to left.


A
B
C
0 m
20 m
40 m
A. Elevation (m) 2.00 1.50 1.00 0.50 0.00
B. Vertical hydraulic gradient -0.22 -0.12 -0.02 0.08 0.18
C. Specific discharge (cm/s) -0.06 -0.04 -0.01 0.01 0.04

of habitat factors (i.e., temperature, intragravel conditions, ice conditions) that may directly affect an integrated process of spawning habitat selection.

Geomorphic controls on hyporheic exchange

Our results demonstrate that the integration of hydrology and geomorphology across spatial scales plays a critical role in structuring hyporheic exchange patterns. Within a drainage, we observed that bounded alluvial valley segments and their associated hyporheic zones were structured longitudinally like beads on a string (sensu Stanford and Ward 1993). Among the study drainages, the amount of groundwater discharge to a stream appeared to vary closely with the size and frequency of bounded alluvial valley segments. The presence of knickpoints, particularly on the downstream end of alluvial valley segments, was critical to the constraining of hyporheic flow. The importance of alluvial floodplain segments and knickpoints to ecosystem structure and function has been observed in large montane rivers (Stanford and Ward 1993). Our results are consistent with the concept that these landforms play a significant role in structuring hyporheic exchange in small tributary systems as well.

Within tributary drainages of the Swan basin, we found that alluvial valley segment types differed in their hyporheic exchange character depending on whether they were bounded or unbounded by a downstream knickpoint. Bounded alluvial valley segments had the most variation in hyporheic exchange and the strongest upwelling zones. Moreover, the greater the spatial extent of a bounded alluvial valley segment, the greater the strength of groundwater input was within the segment. Unbounded alluvial valley segments were always associated with a tributary's confluence with the Swan River and were characterized by reaches with strongly negative VHG. These contrasts and associations are evidence that geomorphic constraint on the length, lateral complexity, volume of alluvial fill, and character of geomorphic knickpoints in alluvial valleys affects hyporheic exchange.

Reaches with upwelling hyporheic groundwater were almost exclusively located within bounded alluvial valley segments. Within a bounded alluvial valley segment, reaches at the upstream end were usually downwelling, and reaches at the downstream end of the floodplain just upstream of the confining knickpoint were upwelling. These sites were also marked by narrowing valley widths partly because the valley walls of bounded alluvial valley segments typically begin to pinch together as the downstream knickpoint is reached. These patterns are consistent with what might be expected based on the application of basic principles of hydrogeology (Freeze and Cherry 1979) at the scale of an alluvial valley segment. Although reaches located at the downstream ends of bounded alluvial valley segments were gaining subsurface water, these reaches were not the only ones with groundwater discharge. In part, we attribute this to nonhyporheic inputs (e.g., seeps from bedrock fissures) that were independent of hyporheic flow pathways. We also observed relatively impermeable clay layers that probably contributed to localized upwelling (i.e., positive VHG) measurements in some instances. In addition, in Cold Creek, we observed negative VHG above a large beaver dam complex and positive VHG for several hundred metres downstream, a phenomenon that has been observed by others (e.g., White 1990).

Within reaches, we also observed associations between geomorphology and hyporheic exchange. We observed that VHG varied significantly with bedform character, as has been shown by others (e.g., Vaux 1968; Thibodeaux and Boyle 1987; Harvey and Bencala 1993). Convex and transitional bedforms displayed localized, shallow downwelling, while concave areas possessed upwelling. In several instances, large wood appeared to create local variation in bed topography and heterogeneity in the magnitude and direction of VHG, where shallow downwelling occurred above logs and upwelling occurred in the pools below logs.

The K (as measured using falling head tests in piezometers) and intragravel v (estimated via Darcy's equation) were highly variable within stream reaches. Transitional bedforms located at the tailout of pools (i.e., just upstream of riffles) possessed the highest values of K and v. This observation concurs with laboratory flume experiments (Cooper 1965; Thibodeaux and Boyle 1987) in which the fastest intragravel flow rates were observed at the crests of waveforms as a consequence of pressure gradients. Qualitative observation of piezometer screens indicated the patchy distribution of clay or fine sediment at varying depths. Thus, both the shape of the stream bed and the structure of the stream substratum may contribute to the spatial variability in intragravel flow. In addition, although we did not investigate conditions within redds, the construction of redds may alter very localized patterns of VHG and may positively influence the K of the disturbed site (Cooper 1965).

At the drainage scale, the arrangement of bounded alluvial valley segments may influence the cumulative thermal and flow regimes of a stream. Although our study did not explicitly address this hypothesis, we did observe that drainages with larger, more frequent bounded alluvial valley segments possessed more stream habitat that was ice free during the winter. The strength and dissipation of the groundwater plume leaving the lower end of a bounded alluvial valley segment may affect the discharge and thermal regimes of areas downstream. We observed that areas influenced by groundwater discharge possessed relatively stable thermal regimes. Furthermore, reaches influenced by upwelling groundwater had less winter ice cover and experienced no anchor ice formation. This was in contrast with frequent occurrence of anchor ice in neutral and losing reaches that were not thermally buffered by upstream groundwater discharge.

Bull trout spawning patterns

The size and distribution of bounded alluvial valley segments on the landscape influence the distribution and abundance of bull trout spawning. In the Swan basin, bull trout redds were most abundant in drainages with extensive bounded alluvial valley segments. Previous landscape-scale analyses of bull trout spawning have suggested there is a significant association between redd frequency and drainage area and (or) stream order (Graham et al. 1981). These conventional drainage metrics may be important discriminators for presence–absence of bull trout spawning at broad spatial scales, as was suggested by Rieman and McIntyre (1995). However, they do not appear to be useful predictors of between-tributary variation in distribution or abundance among the principle spawning streams of the Swan basin.

We found that bull trout selected reaches for spawning with zones of hyporheic groundwater discharge. We also observed that these reaches consistently occurred within bounded alluvial valley segments. Various reach-scale geomorphic factors, such as stream gradient, have been identified as important factors affecting bull trout spawning habitat (Graham et al. 1981; Fraley and Shepard 1989). We found that low stream gradient was a character of both bounded and unbounded alluvial valley segments. Consequently, it was not a direct indicator of spawning reach selection. Rather, bull trout used reaches with hyporheic groundwater discharge that also possessed low stream gradient as a covariate.

At drainage and valley segment scales, the importance of hyporheic exchange and its influence on bull trout spawning may be related to temperature and flow regimes. Numerous researchers have identified alluvial valley segments as critical spawning habitat for many fish species (e.g., Copp 1989; Montgomery et al. 1999). In addition to habitat influenced by groundwater discharge, these segments contain large, stable expanses of well-sorted gravel and cobble. Furthermore, the presence of floodplain surfaces in these segments allows overbank flow, which moderates sediment scour associated with flooding. In contrast, suitable spawning gravel is sparse and individual patches are often small in confined valley segments where floodwaters are not attenuated by a floodplain.

Habitat-use and spawning patterns of bull trout are influenced by the geologic legacy of a basin. Longitudinally stepped drainage forms, bowl-like floodplain shapes, deep alluvial fill, high gravel porosity, and position of knickpoints are features influenced by glaciation. These features create deep hyporheic flow pathways and influence bull trout spawning habitat in the Swan basin. In contrast, hyporheic exchange in similar-sized, nonglaciated stream systems of northeastern Oregon appears to occur only at shallow, small spatial scales (C. Baxter, Oregon State University, Corvallis, Oreg., unpublished data). Thus, many bull trout streams lack the deep, extensive flow pathways typical of glaciated basins and their floodplains. As a consequence of these differences, it is important to consider groundwater – surface water interactions (Creuze des Chatelliers et al. 1994) and bull trout habitat-use patterns in their biogeoclimatic context.

Reaches influenced by groundwater discharge possess relatively stable thermal and flow regimes that may be important for bull trout egg incubation, emergence success, and the survival of juvenile bull trout. Differential survival at any of these stages could influence the distribution of adult spawning. In addition, homing by bull trout may be guided by cues that are related to the unique chemical signature of groundwater. Although little is known about groundwater effects on bull trout, numerous studies have demonstrated the importance of groundwater discharge zones to a related species, the brook trout (*Salvelinus fontinalis*), and other salmonids. For example, our observations of bull trout reflect Benson's (1953) finding that groundwater-influenced reaches of the Pigeon River in Michigan had less ice cover and appeared to be selected as spawning reaches by brook trout. In general, groundwater discharge sources provide cold-water refugia for fish in summer (Gibson 1966; Nielson et al. 1994) and warmwater refugia in winter (Craig and Poulin 1975; Cunjak and Power 1986). In addition, warm groundwater temperatures in winter inhibit the formation of anchor ice, which would otherwise cause high mortality in postemergent salmonids (Benson 1955).

At the pool–riffle spatial scale, we found that bull trout redds were associated with transitional bedforms at the tailouts of pools that formed at the heads of riffles. These sites also possessed localized downwelling (i.e., negative VHG) and high intragravel flow rates. Similarly, in a spawning tributary of the North Fork of the Flathead River, Weaver and White (1985) detected greater permeability in a bull trout spawning area than in a nonspawning area. Whether it is the direction or the magnitude of hyporheic exchange, or both, that is actually involved in redd site selection, we are unsure. Intragravel flow has long been recognized by fisheries biologists as important to the growth, development, and survival of salmonid eggs and fry. Numerous salmonid species tend to spawn in the transitional zone between pools and riffles where intragravel flow rates are high, and percolation through the redd is thought to provide a constant supply of oxygen to the eggs and effectively remove metabolic waste materials (Bjornn and Reiser 1991). Finer spatial resolution in measures of VHG and intragravel flow rate, along with quantification of other factors such as substrate character and intragravel dissolved oxygen levels, would further delineate the relative importance of hyporheic exchange to spawning site selection at the microhabitat scale. Study of bull trout sensory capabilities, along with their growth and survival to emergence and the success of rearing juveniles in habitats possessing variable hyporheic exchange characters, could shed more light on the underlying biological mechanisms of these habitat-use patterns.

It appears that there is a spatially nested relationship between hyporheic exchange and bull trout spawning habitat use. Although we found at the larger spatial scales that bull trout spawning was associated with zones of positive VHG, at the pool–riffle scale, we found bull trout redd distributions associated with localized downwelling (i.e., negative VHG) and transitional bedforms between pools and riffles. Without a hierarchical approach to the interaction between hyporheic exchange and bull trout spawning, we would have obtained an erroneous picture of spawning habitat selection. For example, if we had only examined hyporheic exchange in the immediate vicinity of redds, we might have concluded that spawning habitat selection occurred within downwelling areas and had nothing to do with upwelling groundwater. Alternatively, had we ignored the smaller scale, the unique associations that we observed between VHG, transitional bedforms, intragravel flow, and specific redd placement would have been missed.

Much of the fisheries research on groundwater effects has focused on characteristics of the environment in and immediately surrounding redds (e.g., Webster and Eriksdottir 1976; Curry and Noakes 1995) and the consequences for growth and survival to emergence in redds with varying rates of subsurface flow (e.g., Sowden and Power 1985). The findings of this study suggest the drainage and valley segment scale context of a spawning site or a spawning reach are important considerations. Variation of some habitat factors may not be detectable or important to bull trout at all scales. Hyporheic exchange, however, appears to be important to spawning habitat selection processes at each of the spatial scales examined. In much of the fisheries research attempting to characterize habitat use, habitat variables have been considered as describing local conditions, regardless of their primary scales of heterogeneity. As we and other authors (e.g., Poizat and Pont 1996; Torgersen et al. 1999) have observed, this approach may obscure meaningful patterns by mixing specific organismal responses to habitat factors that vary differently at different scales.

The findings of this study emphasize the importance of considering local geomorphic context and spatial scale when describing stream processes. These factors are treated incompletely within the existing paradigm of the river continuum concept (Vannote et al. 1980; Minshall et al. 1985). Rather, our observations of the importance of local variability in hydrogeomorphic processes to habitat use by spawning bull trout may represent an example of the applicability of Montgomery's (1999) process domain concept. A landscape context is important to a better understanding of hyporheic processes, as geomorphology plays a scale-specific role in patterning variation of hyporheic exchange. Many studies of hyporheic processes have been done within a single, short stream reach (e.g., Harvey and Bencala 1993). Our observations demonstrate that the valley segment and drainage scale context of a reach could influence the findings of such a study and that quantifying hyporheic exchange in a spatially extensive manner is both critical and feasible.

Conserving the bull trout and other fish species that possess complex migratory life histories demands a landscape perspective (Schlosser 1995). Our findings demonstrate that the conservation of bull trout and the maintenance and (or) restoration of spawning habitat will require consideration of groundwater – surface water exchange within a hierarchical, geomorphic context. For example, the distribution of bounded alluvial valley segments on the landscape may be significant to bull trout restoration plans in this region because of the importance of this distribution to understanding the spatial geometry of bull trout habitat (e.g., Rieman and McIntyre 1995; Dunham and Rieman 1998). This study has helped to identify one component of the functional combination of habitats in the landscape mosaic required by this species. Finally, these findings have contributed to the growing body of research demonstrating the importance of hyporheic processes to the structure and function of stream ecosystems.

Acknowledgements

We thank Jack Stanford, Chris Frissell, Bill Woessner, and Bonnie Ellis for their thoughtful insights and discussions throughout this research project. The senior author thanks fellow graduate students and staff at the Flathead Lake Biological Station for their support and sharing of ideas. The bull trout redd data were graciously provided by Tom Weaver of the Montana Department of Fish, Wildlife and Parks. We thank Geoff Poole for his assistance in data analyses. Special thanks go to Laura Weaver-Baxter and Joe Giersch for valuable help with field data collection and to Christian Torgersen for helpful review of the manuscript. We also extend our gratitude to several landowners that provided access to streams on their properties. This study was supported by funding from the National Science Foundation EPSCoR program, Montana Department of Fish, Wildlife and Parks, and the Biological Resources Division of the U.S. Geological Survey, Global Climate Change program.

References

Baxter, C.V., Frissell, C.A., and Hauer, F.R. 1999. Geomorphology, logging roads, and the distribution of bull trout spawning in a forested river basin: implications for management and conservation. Trans. Am. Fish. Soc. **128**: 854–867.

Bayley, P.B., and Li, H.W. 1992. Riverine fishes. *In* The rivers handbook: hydrological and ecological principles. *Edited by* P. Calow and G.E. Petts. Blackwell, Oxford, U.K. pp. 251–281.

Benson, N.G. 1953. The importance of groundwater to trout populations in the Pigeon River, Michigan. Trans. N. Am. Wildl. Nat. Resour. Conf. **18**: 260–281.

Benson, N.G. 1955. Observations on anchor ice in a Michigan trout stream. Ecology, **36**: 529–530.

Bjornn, T.C., and Reiser, D.W. 1991. Habitat requirements of salmonids in streams. *In* Influences of forest and rangeland management on salmonid fishes and their habitats. *Edited by* W.R. Meehan. Am. Fish. Soc. Spec. Publ. No. 19. pp. 83–138

Boulton, A.J., Findlay, S., Marmonier, P., Stanley, E.H., and Valett, H.M. 1998. The functional significance of the hyporheic zone in streams and rivers. Annu. Rev. Ecol. Syst. **29**: 59–81.

Brunke, M., and Gonser, T. 1997. The ecological significance of exchange processes between rivers and groundwater. Freshwater Biol. **37**: 1–33.

Cedergren, H.R. 1989. Seepage, drainage, and flow nets. John Wiley & Sons, New York.

Church, M. 1992. Channel morphology and typology. *In* The rivers handbook: hydrological and ecological principles. *Edited by* P. Calow and G.E. Petts. Blackwell, Oxford, U.K. pp. 126–143.

Cooper, A.C. 1965. The effect of transported stream sediments on the survival of sockeye and pink salmon eggs and alevins. Int. Pac. Salmon Fish. Comm. Bull. No. 18.

Copp, G.H. 1989. The habitat diversity and fish reproductive function of floodplain ecosystems. Environ. Biol. Fishes, **26**: 1–27.

Craig, P.C., and Poulin, V.A. 1975. Movements and growth of arctic grayling (*Thymallus arcticus*) and juvenile arctic char (*Salvelinus alpinus*) in a small arctic stream, Alaska. J. Fish. Res. Board Can. **32**: 689–697.

Creuze des Chatelliers, M., Poinsart, D., and Bravard, J.P. 1994. Geomorphology of alluvial groundwater ecosystems. *In* Groundwater ecology. *Edited by* J. Gibert, D.L. Danielopol, and J.A. Stanford. Academic Press, San Diego, Calif. pp. 477–500.

Cunjak, R.A., and Power, G. 1986. Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. **43**: 1970–1981.

Curry, R.A., and Noakes, D.L.G. 1995. Groundwater and the selection of spawning sites by brook trout (*Salvelinus confluentus*). Can. J. Fish. Aquat. Sci. **52**: 1733–1740.

Dunham, J.B., and Rieman, B.E. 1998. Metapopulation structure of bull trout: influences of physical, biotic, and geometrical landscape characteristics. Ecol. Appl. **9**(2): 642–655.

Dutilleul, P., and Legendre, P. 1993. Spatial heterogeneity against heteroscedasticity: an ecological paradigm versus a statistical concept. Oikos, **66**: 152–171.

Fraley, J., and Shepard, B. 1989. Life history, ecology and population status of migratory bull trout (*Salvelinus confluentus*) in the Flathead Lake and River system, Montana. Northwest Sci. **63**: 133–143.

Freeze, R.A., and Cherry, J.A. 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Frissell, C.A., Liss, W.J., Warren, C.E., and Hurley, M.D. 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. Environ. Manage. **10**: 199–214.

Gibson, R.J. 1966. Some factors influencing the distribution of brook trout and young Atlantic salmon. J. Fish. Res. Board Can. **23**: 1977–1979.

Graham, P.J., Shepard, B.B., and Fraley, J.J. 1981. Use of stream habitat classifications to identify bull trout spawning areas in streams. *In* Acquisition and utilization of aquatic habitat inventory information. *Edited by* N.B. Armantrout. Western Division of the American Fisheries Society, Portland, Oreg. pp. 186–191.

Gregory, S.V., Swanson, F.J., McKee, W.A., and Cummins, K.W. 1991. An ecosystem perspective of riparian zones. BioScience, **41**: 540–551.

Grimm, N.B., and Fisher, S.G. 1984. Exchange between surface and interstitial water: implications for stream metabolism and nutrient cycling. Hydrobiologia, **111**: 219–228.

Harvey, J.W., and Bencala, K.E. 1993. The effect of streambed topography on surface–subsurface water exchange in mountain catchments. Water Resour. Res. **29**: 89–98.

Hvorslev, M.J. 1951. Time lag and soil permeability in groundwater observations. Bull. No. 36, Waterways Experiment Station, Corps of Engineers, Vicksburg, Miss.

Hynes, H.B.N. 1975. The stream and its valley. Verh. Int. Ver. Theor. Angew. Limnol. **19**: 1–15.

Jacobs, J. 1974. Quantitative measurement of food selection; a modification of the forage ratio and Ivlev's electivity index. Oecologia, **14**: 413–417.

Junk, W.J., Bayley, P.B., and Sparks, R.E. 1989. The flood pulse concept in river–floodplain systems. *In* Proceedings of the International Large River Symposium. *Edited by* D.P. Dodge. Can. Spec. Publ. Fish. Aquat. Sci. No. 106. pp. 110–127.

Kleinkopf, M.D., Harrison, J.E., and Zartman, R.E. 1972. Aeromagnetic and geologic map of part of northwestern Montana and northern Idaho: U.S. Geological Survey Geophysical Investigations Map GP-830. U.S. Geological Survey, Washington, D.C.

Leathe, S.A., and Enk, M.D. 1985. Cumulative effects of microhydro development on the fisheries of the Swan River drainage, Montana. Vol. I. Summary report. Project No. 82-19. Montana Department of Fish, Wildlife and Parks, Kalispell, Mont.

Lee, D.R., and Cherry, J.A. 1978. A field exercise on groundwater flow using seepage meters and mini-peizometers. J. Geol. Educ. **27**: 6–10.

Minshall, G.W., Cummins, K.W., Peterson, R.C., Cushing, C.E., Bruns, D.A., Sedell, J.R., and Vannote, R.L. 1985. Developments in stream ecosystem theory. Can. J. Fish. Aquat. Sci. **42**: 1045–1055.

Montgomery, D.R. 1999. Process domains and the river continuum. J. Am. Water Res. Assoc. **35**: 397–410.

Montgomery, D.R., Abbe, T.B., Buffington, J.M., Peterson, N.P., Schmidt, K.M., and Stock, J.D. 1996. Distribution of bedrock and alluvial channels in forested mountain drainage basins. Nature (Lond.), **381**: 587–589.

Montgomery, D.R., Beamer, E.M., Pess, G.R., and Quinn, T.P. 1999. Channel type and salmonid spawning distribution and abundance. Can. J. Fish. Aquat. Sci. **56**: 377–387.

Nielson, J.L., Lisle, T.E., and Ozaki, V. 1994. Thermally stratified pools and their use by steelhead in northern California streams. Trans. Am. Fish. Soc. **123**: 613–626.

Northcote, T.G. 1978. Migratory strategies and production in freshwater fishes. *In* Ecology of freshwater fish production. *Edited by* S.D. Gerking. Blackwell, Oxford, U.K. pp. 326–359.

Poizat, G., and Pont, D. 1996. Multi-scale approach to species–habitat relationships: juvenile fish in a large river section. Freshwater Biol. **36**: 611–622.

Rieman, B.E., and McIntyre, J.D. 1993. Demographic and habitat requirements for conservation of bull trout. U.S. For. Serv. Intermountain Res. Stn. Gen. Tech. Rep. INT-308.

Rieman, B.E., and McIntyre, J.D. 1995. Occurrence of bull trout in naturally fragmented habitat patches of varied size. Trans. Am. Fish. Soc. **124**: 285–296.

Schlosser, I.J. 1995. Critical landscape attributes that influence fish population dynamics in head water streams. Hydrobiologia, **303**: 71–81.

Sowden, T.K., and Power, G. 1985. Prediction of rainbow trout embryo survival in relation to groundwater seepage and particle size of spawning substrates. Trans. Am. Fish. Soc. **114**: 804–812.

Stanford, J.A., and Ward, J.V. 1988. The hyporheic habitat of river ecosystems. Nature (Lond.), **335**: 64–66.

Stanford, J.A., and Ward, J.V. 1993. An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. J. North Am. Benthol. Soc. **12**: 48–62.

Strahler, A.N. 1964. Quantitative geomorphology of drainage basins and channel networks. *In* Handbook of applied hydrology. *Edited by* Ven te Chow. McGraw-Hill, New York.

Swanson, F.J., Kratz, T.K., Caine, N., and Woodmansee, R.G. 1988. Landform effects on ecosystem patterns and processes. BioScience, **38**: 92–98.

Terhune, L.D.B. 1958. The Mark VI standpipe for measuring seepage through salmon spawning gravel. J. Fish Res. Board Can. **15**: 1027–1063.

Thibodeaux, L.J., and Boyle, J.D. 1987. Bedform-generated convective transport in bottom sediment. Nature (Lond.), **325**: 341–343.

Torgersen, C.E., Price, D.M., Li, H.W., and McIntosh, B.A. 1999. Multiscale thermal refugia and stream habitat associations of chinook salmon in northeastern Oregon. Ecol. Appl. **9**: 327–345.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., and Cushing, C.E. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. **37**: 130–137.

Vaux, W.G. 1968. Intragravel flow and interchange of water in a streambed. U.S. Fish Wildl. Serv. Fish. Bull. **66**: 479–489.

Weaver, T.M., and White, R.G. 1985. Coal Creek fisheries monitoring study number III. Contract No. 53-0385-3-2685. U.S. Forest Service Flathead National Forest, Kalispell, Mont.

Webster, D.A., and Eiriksdottir, G. 1976. Upwelling as a factor influencing choice of spawning sites by brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. **105**: 416–421.

White, D.S. 1990. Biological relationships to convective flow patterns within stream beds. Hydrobiologia, **196**: 149–158.

White, D.S. 1993. Perspectives on defining and delineating hyporheic zones. J. North Am. Benthol. Soc. **12**: 61–69.

Wiens, J.A. 1989. Spatial scaling in ecology. Funct. Ecol. **3**: 385–397.

Influence of body weight, swimming characteristics, and water temperature on the cost of swimming in brook trout (*Salvelinus fontinalis*)

Ming Tang, Daniel Boisclair, Chantal Ménard, and John A. Downing

Abstract: We performed respirometry experiments to estimate the spontaneous swimming costs of brook trout (*Salvelinus fontinalis*) for 24 combinations of fish weight (3.5, 17, and 32 g), water temperature (4, 12, and 18°C), and respirometer size (27, 54, and 108 L). Fish swimming characteristics were estimated for each experiment using videocamera recordings and image analysis. Under our experimental conditions, average swimming characteristics of fish, such as swimming speed and turning and acceleration rates, varied from 2.5- to 29-fold. Our data, alone or combined with similar published results on brook trout weighing 1 g, indicated that fish weight was the only variable that could explain a statistically significant proportion of the variations of spontaneous swimming costs for that species ($r^2 = 0.91$). Our work confirms, with a wider range of experimental data, that spontaneous swimming costs of fish are 3- to 22-fold (8-fold average difference) more energy demanding than predicted by forced swimming models developed using fish swimming at constant speeds and directions in flumes.

Résumé : Nous avons réalisé des expériences respirométriques visant à estimer les coûts de la nage spontanée de l'omble de fontaine (*Salvelinus fontinalis*) pour 24 combinaisons de masse des poissons (3,5, 17 et 32 g), de température de l'eau (4, 12 et 18°C) et de taille de respiromètres (27, 54 et 108 L). Les caractéristiques de nage des poissons ont été estimées, pour chaque expérience, à l'aide d'enregistrements par vidéocaméras et d'analyses d'images. Sous nos conditions expérimentales, les caractéristiques de nage moyennes dont la vitesse de nage, les taux de virage et d'accélération ont varié par un facteur de 2,5 à 29. Nos données, seules ou combinées avec des résultats similaires publiés pour des ombles de fontaine de 1 g, ont indiqué que la masse des poissons est la seule variable qui peut expliquer une proportion statistiquement significative des coûts de la nage spontanée pour cette espèce ($r^2 = 0{,}91$). Nos travaux confirment, avec une gamme plus étendue de données expérimentales, que les coûts de la nage spontanée des poissons sont 3 à 22 fois (8 fois en moyenne) plus élevés que ceux prédits par les modèles de la nage forcée développés avec des poissons nageant à des vitesses et dans des directions constantes.

Introduction

Estimation of fish swimming costs is of key importance for many ecological studies. Models and variables that represent the energetic expenditures associated with movements performed by fish to feed, migrate, and escape predators are inherent components of optimal foraging, habitat quality, and bioenergetic models (Ware 1975; Kitchell et al. 1977; Hughes and Dill 1990). The importance of the cost of swimming in ecological studies is further illustrated by the number of approaches developed to estimate fish movements under field situations (radiotracking of fish position: Holliday et al. 1974; estimation of the frequency of contractions of axial muscles or estimation of tail beat frequency: Ross et al. 1981; Weatherley et al. 1982; video observations: Ménard 1991; Boisclair 1992*a*). All approaches that attempt to transform fish movements into swimming costs require equations that describe the relationship between energy expenditures, fish attributes (fish weight, muscular or swimming activity), and environmental variables (water temperature). Consequently, the validity of these approaches and equations determines the quality of the predictions made by a wide range of ecological models.

Fish swimming costs have long been presumed to increase with swimming complexity (Beamish and Dickie 1967). Empirical models developed by Boisclair and Tang (1993) provided a quantitative framework for formalizing this expectation. These models showed that fish weight and average speed could explain a large fraction of swimming costs ($0.76 < R^2 < 0.80$). Furthermore, they suggested that, for specific combinations of fish weight and average speed, the cost of swimming for fish allowed to change their speed and direction (spontaneous swimming in a rectangular aquarium)

Received October 5, 1999. Accepted March 16, 2000.
J15388

M. Tang, D. Boisclair,[1] C. Ménard, and J.A. Downing. Département de sciences biologiques, Université de Montréal, CP 6128, Succursale Centre-ville, Montréal, QC H3C 3J7, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: daniel.boisclair@umontreal.ca

may be 6–14 times more expensive than for fish forced to swim at a unidirectional and constant speed (forced swimming in a flume).

The magnitude of the difference between spontaneous and forced swimming costs has been attributed to the added cost of performing accelerations and turns (Webb 1991; Boisclair and Tang 1993). This led to the hypothesis that swimming characteristics other than speed could contribute to explaining the variation in spontaneous swimming costs (Krohn and Boisclair 1994). This hypothesis, however, could not be tested using the original data set gathered by Boisclair and Tang (1993) because swimming speed was the only swimming characteristic available. Experimental evidence combining respirometry data and detailed assessments of swimming characteristics of brook trout (*Salvelinus fontinalis*) confirmed predictions of the magnitude of the difference in the energetic cost between spontaneous and forced swimming (Krohn and Boisclair 1994; Tang and Boisclair 1995). These analyses also showed that variables such as the variance in speed and the average turning rate may explain a fraction of the variation in spontaneous swimming costs equivalent to, and sometimes larger than, average speed. However, these experiments focused on relatively small brook trout (1.2 g wet weight) with few experiments performed on brook trout of 5–10 g wet weight. Despite the expected influence of fish weight on metabolism (Brett and Glass 1973; Kitchell et al. 1977; Beamish 1978; Boisclair and Tang 1993), no data are currently available to assess the relative effect of fish weight and a complete suite of swimming characteristics on spontaneous swimming costs. Furthermore, no experimental data have been produced to test the predictions made by the model proposed by Boisclair and Tang (1993) for different fish size-classes.

The objectives of our study were to (*i*) evaluate the relative influence of fish weight, swimming characteristics, and water temperature on spontaneous swimming costs of brook trout and (*ii*) contribute to the development of a more powerful and general model that would allow the quantification of the cost of spontaneous movements for fishes with a carangiform or subcarangiform swimming mode.

Methods

Experimental design

The influence of water temperature, fish body mass, and swimming characteristics on the cost of spontaneous swimming was quantified by measuring oxygen consumption for fish of different sizes kept at three water temperatures and under conditions that induced them to display different swimming characteristics. This was achieved by performing 24 respirometry experiments with juvenile brook trout having a mean weight of 3.5 (SD = 0.4), 17.3 (SD = 6.1), and 32.2 (SD = 8.2) g wet at water temperatures of 4, 12, and 18°C using respirometers of 27 (30 × 30 × 30 cm), 54 (60 × 30 × 30 cm), and 108 L (120 × 30 × 30 cm). This approach was adopted because the volume available for swimming has a significant influence on fish swimming characteristics (Tang and Boisclair 1993; Krohn and Boisclair 1994). Each experiment was one combination of a water temperature and a respirometer size except for the largest fish, which were not tested in the 27-L respirometer because it severely restricted fish movements (longest dimension of the respirometer <1.5 fish body length). Fish swimming characteristics (speed, acceleration, and turning rates) were estimated using the stereocinematographic method (Ménard 1991; Boisclair 1992*a*, 1992*b*). Young-of-the-year brook trout were selected as the subject of our work because individuals from a standard size and homogenous inbred strain were easily obtained from a fish farm (Réserve La Petite Nation, Quebec). Furthermore, this permitted the combination of the results of the present study with those of similar experiments performed in our laboratory using brook trout averaging 1.2 (SD = 0.15) g (Tang and Boisclair 1995).

Experimental procedure

Estimation of spontaneous swimming costs

Fish were acclimatized to a given water temperature using a 500-L Living Stream^{TM} aquarium (model LSW 700). During acclimation, water temperature was increased or decreased 1°C·day^{-1} until it reached the desired level. Fish were maintained at that temperature for more than 14 days prior to an experiment. For experiments performed at 4°C, fish were fasted for 4 days prior to an experiment to avoid the confounding effects of digestive processes on respiration (Brett 1964; Brett and Groves 1979). The fasting period was set at 3 days for temperatures at 12°C and at 2 days at 18°C.

The respirometers consisted of Plexiglas boxes with lids. Three sides of the respirometers were covered with black tape to reduce outside disturbance and to improve fish observability. The respirometers were placed in a second LSW 700 to ensure precise control of water temperature during the experiments. Two water pumps were installed in the respirometers: pump A was used for mixing and maintaining a homogenous oxygen concentration within the respirometer during an experiment, and pump B allowed a connection between the inside and outside of the respirometers.

Four young-of-the-year brook trout were transferred from the acclimation tank to their respective respirometer 24 h before the onset of an experiment (hereafter referred to as the stabilization period). The number of fish used in our experiments was selected to ensure that significant respiration (considering the precision of our oxygen meter (Intab 565, ±0.005 ppm)) could be estimated for all fish sizes and water temperatures tested and for all volumes of aquaria employed. Because agonistic behaviour could unduly increase estimates of respiration rates, experiments were interrupted when persistent agonistic behaviour was observed. Following the introduction of fish into the respirometer, it was sealed without residual air bubbles. During the stabilization period, pump B generated a small exchange between the respirometer and the holding tank. This circulation of water maintained 75–80% oxygen saturation inside the respirometer. At the beginning of an experiment, pump B was connected to the oxymeter, thereby preventing new water from leaking into the respirometer from the holding tank. Initial oxygen concentration was measured using two steps. First, pump A was turned on for 1 min to homogenize oxygen concentration inside the respirometer. Second, pump B transferred water from the respirometer to the probe of the oxymeter and back to the respirometer. Water from the respirometer never contacted air during this process. Additional measurements of oxygen concentration were taken every 30–60 min using this same procedure. Pump A was not turned on between oxygen measurements in order to minimize fish disturbance.

An experiment was terminated when fish had consumed more than 0.5 ppm oxygen or after 12 h of observation. No observations were made below oxygen concentrations of 7.6 ppm. This limit was imposed to minimize the influence of low oxygen concentration on fish behaviour. Water temperature never varied by more than 0.5°C during an experiment. At the end of an experiment, fish were immediately removed from the respirometer and their weight (±0.005 g wet) and total length (±0.05 cm) measured individually. Biological oxygen demand (BOD) inside the respirometer filled with water was determined within 24 h of each experiment using the same procedure as described above but without fish.

Estimation of swimming characteristics

Fish swimming characteristics were estimated from the temporal variation of fish positions in an *x,y,z* Cartesian coordinate system. This procedure required the filming and computer analysis of the movements executed by fish during each experiment.

Fish movements in the respirometers were filmed with a pair of videocameras (Panasonic WV-BL602) through a glass window (0.3 × 1.5 m) located on the side of the LSW 700. The cameras were oriented in parallel and separated by 10 cm. Images obtained by the two cameras were synchronized and combined side by side into a single image by a videoprocessor (Panasonic Quad WJ-450). Time of recording was printed by a time and date camera titler (Panasonic WJ-810) precise to ±0.005 s. The final images were sent to a videocassette recorder (Sony EV-C3). Fish were filmed 10–50 $\text{min}\cdot\text{h}^{-1}$ depending on the duration of the experiment (2.5–12 h). We recorded a total of 120 min of film per experiment. Photoperiod during our experiments ranged from 16 h light : 8 h dark to 14 h light : 10 h dark. Experiments were performed only during the lighted period of the day. During the lighted period of the day, two fluorescent lights located above the respirometers were turned on to homogenize lighting conditions and to improve the quality of the images.

Images recorded were analyzed using the Jandel Video Analysis Software (Java). Calibrations necessary to transform images filmed in fish *x,y,z* positions were performed using the method described by Boisclair (1992*a*, 1992*b*) and Krohn and Boisclair (1994). The position of a fish was quantified by four pairs of coordinates (the *x* and the *y* coordinates of the head and tail of the fish, respectively). For each experiment, we selected 50 sequences of 10 s of filming. These sequences were distributed at random over the complete duration of an experiment. For each sequence, individual fish inside the respirometer were classified as swimming or resting. The resting mode was assigned to fish moving less than 0.5 $\text{cm}\cdot\text{s}^{-1}$ for 3 consecutive seconds. The coordinates of one fish defined as swimming during a complete sequence was estimated at 1-s intervals during 10 successive seconds. This procedure allowed us to estimate 10 successive fish positions per sequence and 500 fish positions for each experiment.

Computations

Estimation of spontaneous swimming costs

Spontaneous metabolism (C_R, milligrams oxygen) of an average fish during a specific experiment was calculated as

$$(1)\qquad C_R = (D[O_2] - \text{BOD}) \times V/4$$

where $D[O_2]$ is oxygen depletion (milligrams oxygen per litre) during an experiment, BOD is the biological oxygen demand of the water (milligrams oxygen per litre), V is the volume (litres) of the respirometer, and 4 is the number of fish inside the respirometer. Spontaneous swimming costs (C_S, milligrams oxygen per hour) were estimated as

$$(2)\qquad C_S = (C_R - (\text{SMR} \times t))/(t \times P_t)$$

where SMR is the standard metabolic rate (milligrams oxygen per hour) of a fish, t is the duration (hours) of the experiment, and P_t is the proportion of time that fish spent swimming (see eq. 4). Because C_S corresponds to the respiration rate of fish excluding respiration associated with standard metabolism and digestion (fish were fasted), this value is presumed to represent only the cost of swimming (i.e., that of performing external movements).

SMR was determined with a model (eq. 3) that we developed using data from Job (1955) on standard metabolism of brook trout (0.19–54.1 mg $O_2\cdot h^{-1}$) weighing 5–1000 g held at water temperatures ranging from 5 to 20°C:

$$(3)\qquad \log_{10}\text{SMR} = 0.85\log_{10}W + 0.04T - 1.45$$

where W is the mass of the fish (grams wet weight) and T is the water temperature (degrees Celsius). In the experiments performed by Job (1955), the fish were sometimes fasted for as little as 12 h. This protocol may not be sufficient to eliminate oxygen consumption associated with digestion processes. Furthermore, SMRs were defined as the lowest rate of oxygen consumption of fish during any 2-h interval in a 24-h period. The setup used by Job (1955) (described by Fry and Hart 1948) allowed fish to swim freely. This situation implies that the SMR estimates produced by eq. 3 may represent maximum values (including unspecified costs of digestion and spontaneous activity) and consequently that our measures of activity costs should be interpreted as minimum values.

The P_t was estimated as

$$(4)\qquad P_t = \left(\sum_{k=1}^{k} P_k\right)/(k \times 240)$$

where P_k is the total number of seconds that fish were in the swimming mode during 1 min sampled at random from within every 5 min of filming, k is the number of times that P_k was estimated during an experiment (k = 120 min of filming per experiment/5-min intervals = 24), and 240 is the maximum number of seconds that fish can be active per minute (4 fish per respirometer × 60 s per minute). The time available for swimming is here defined as $k \times 240$ (5760 s). When more than one fish was in the swimming mode during a given second, the number of seconds added to P_k was equal to the number of fish swimming.

Swimming characteristics

Fish movements were described using three characteristics: speed, acceleration, and turning rates. Swimming characteristics were estimated from the displacements of a fish in an *x,y,z* coordinate system during a given time period (generally 1–2 s). A complete description of the geometric formulae used to estimate fish swimming characteristics from successive *x,y,z* positions of the tail and head of a fish was presented by Tang and Boisclair (1993).

Data analysis

Spontaneous swimming costs

Comparison of spontaneous swimming costs among experiments was performed using a permutation procedure consisting of four steps. (*i*) A total of 12 000 pseudo-values of spontaneous swimming costs were created for the 24 experiments (further referred to as nominal sets of pseudo-values). Each set of 500 pseudo-values was generated by adding 500 error values to the respiration rate estimated during a specific experiment. Error values were randomly chosen from a normal distribution having a mean of zero and a standard deviation of 0.007. The standard deviation was estimated using the instrumental error (0.005 mg $O_2\cdot L^{-1}$) of the respirometer, the number of oxygen readings necessary to estimate fish respiration (four: two readings for total respiration and two readings for BOD), and the property that over 99% of observations from a normal distribution are within ±3 SD of the mean ($0.005 \times 4/3 = 0.007$). (*ii*) Variations in respiration rates among the 24 sets of pseudo-values were tested using a parametric three-way analysis of variance (ANOVA). The F values were calculated for fish weight, water temperature, and aquarium size. The interaction terms were not tested because it was expected that they would greatly complicate our simulations and have no effect on our interpretation of the existence of significant variations in spontaneous swimming costs among experiments. (*iii*) An additional series of 49 three-way

Table 1. Experimental conditions, proportion of available time that fish were engaged in swimming activity (P_t), and average (subscript a) or variance in (subscript v) swimming characteristics among experiments.

Experiment	T (°C)	V (L)	W (g)	SSC (mg $O_2 \cdot h^{-1}$)	P_t (%)	S_a ($cm \cdot s^{-1}$)	S_v	A_v	TR_a ($° \cdot s^{-1}$)	TR_v
1	4	27	3.79	0.58	44.7	4.18	7.5	4.8	43.2	1715
2	4	54	4.25	0.71	66.3	5.64	12.0	13.1	37.0	1485
3	4	108	4.66	0.89	37.2	5.06	3.7	4.4	26.1	975
4	4	27	13.82	2.07	59.7	8.46	40.4	42.5	54.7	1968
5	4	54	14.26	1.80	29.5	3.79	5.7	4.4	30.6	1442
6	4	108	10.86	5.24	15.8	3.81	4.5	3.9	32.0	1982
7	4	54	34.83	9.12	41.4	8.77	34.6	35.9	48.5	2050
8	4	108	31.19	12.07	14.4	7.05	6.6	4.7	24.6	573
9	12	27	3.87	1.08	57.0	6.79	18.6	26.1	53.9	1949
10	12	54	3.08	1.17	42.7	5.61	17.0	17.1	43.5	1742
11	12	108	3.50	1.37	81.9	6.78	8.6	12.9	43.4	1447
12	12	27	11.20	2.73	40.3	5.22	10.2	7.9	48.7	1946
13	12	54	13.39	5.41	46.1	10.27	74.9	44.7	55.7	2475
14	12	108	13.27	3.74	49.9	13.48	84.0	86.2	54.3	2905
15	12	54	28.40	9.13	19.7	7.01	10.9	17.2	40.1	1628
16	12	108	31.77	20.29	13.7	7.08	18.6	15.1	37.6	2410
17	18	27	3.64	0.71	54.6	8.09	37.5	43.9	63.4	2656
18	18	54	3.70	0.78	89.3	8.88	15.8	19.3	58.6	1819
19	18	108	3.55	1.32	57.5	8.79	22.1	26.8	48.9	1967
20	18	27	14.09	1.65	68.7	7.40	20.1	27.0	60.1	2036
21	18	54	16.42	2.91	77.4	6.64	12.8	11.8	48.8	1860
22	18	108	16.19	2.37	78.6	8.25	21.3	17.4	45.9	2359
23	18	54	40.13	6.62	77.2	9.00	109.3	21.7	60.8	2879
24	18	108	41.56	12.06	68.2	12.81	102.9	87.1	53.3	2710

Note: *T*, water temperature; *V*, aquarium volume; *W*, mean fish weight; SSC, spontaneous swimming costs; *S*, swimming speed; *A*, acceleration; TR, turning rate.

ANOVAs were executed. Each ANOVA was done using 24 reconstructed sets of swimming costs pseudo-values (500 pseudo-values per set). Reconstruction was performed by redistributing 12 000 swimming costs pseudo-values at random into 24 groups. (*iv*) Among-experiment variations were declared significant when the *F* value of the ANOVA performed using the nominal swimming costs pseudo-values was greater than 95% of the 49 *F* values obtained using the reconstructed sets. Similarly, the statistical significance of the influence of fish weight, water temperature, and aquarium size was determined by comparing their *F* values obtained using the nominal swimming costs pseudo-values with the respective sets of 49 *F* values calculated using reconstructed sets.

Swimming characteristics

Among-experiment variations in swimming characteristics were tested using a permutation procedure similar to that used for spontaneous swimming costs but with two differences: first, we did not have to use pseudo-values to perform the tests (the sets of swimming characteristics estimated from fish coordinates were used directly) and, second, the nominal set of data for each experiment contained 190 swimming characteristics instead of 500 spontaneous swimming costs values. Homogeneity of the variance in the swimming characteristics among experiments was assessed using Bartlett's test performed following a permutation procedure (Sirois and Boisclair 1995).Variations in the proportion of time that fish spent in the swimming mode were assessed using a three-way ANOVA without replicates.

Models

The relationships (linear and logarithmic) between spontaneous swimming costs, water temperature, fish weight, and swimming characteristics were developed using stepwise multiple regression analysis. The average and the variance in each swimming characteristic were used as independent variables to explain variations in spontaneous swimming costs (Sokal and Rohlf 1995). One exception to this rule was acceleration rates, since they are always expected to average zero.

Results

Spontaneous swimming costs

Spontaneous swimming costs ranged from 0.58 to 20.29 mg $O_2 \cdot h^{-1} \cdot fish^{-1}$ (Table 1). Spontaneous swimming costs varied with fish weight ($p < 0.02$), water temperature ($p < 0.02$), and respirometer size ($p < 0.02$). The proportion of time used by fish for swimming ranged from 13.7 to 89.3% (Table 1) and varied with temperature ($p = 0.0028$) but was independent of fish weight ($p = 0.19$) and respirometer size ($p = 0.81$).

Swimming characteristics

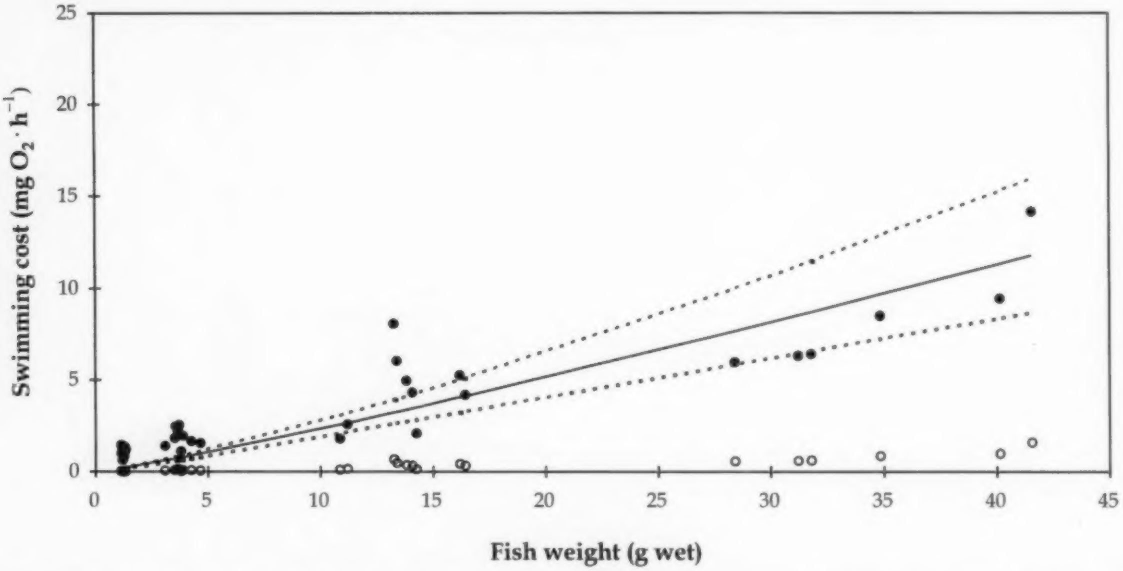
Average swimming speed ranged from 3.79 to 13.48 $cm \cdot s^{-1}$ (Table 1). Fish swimming speed varied with fish weight ($p < 0.02$), water temperature ($p < 0.02$), and respirometer size ($p < 0.02$) and tended to increase with water temperature ($p < 0.01$).

Average turning rate ranged from 24.56 to 63.39$° \cdot s^{-1}$ (Table 1) and tended to increase with water temperature ($p < 0.0002$) and decrease with respirometer size ($p < 0.0009$). Average turning rate was significantly influenced by fish weight ($p < 0.02$), water temperature ($p < 0.02$), and respirometer size ($p < 0.02$).

Table 2. Models to estimate spontaneous swimming costs (C, mg $O_2 \cdot h^{-1}$) of brook trout using fish weight (*W*, g wet) as an independent variable.

Model	r^2	*p*	*n*
Our study			
Allometric model: $\log_{10}C = 1.035\log_{10}W - 0.645$	0.81	<0.0001	24
Linear model			
$C = 0.319W - 0.453$	0.66	<0.0001	24
$C = 0.265W - 0.120$	0.79	<0.0001	23
Combination of our study and data from Tang and Boisclair (1995)			
Allometric model: $\log_{10}C = 1.131\log_{10}W - 0.759$	0.91	<0.0001	42

Fig. 1. Comparison between the predictions made by the allometric model that we developed (solid line; broken lines are 95% CI) by combining our data with those of Tang and Boisclair (1995) and predictions of spontaneous (solid circles) and forced swimming (open circles) using the models of Boisclair and Tang (1993).



The variances of speed (3.67–109.33), acceleration rates (3.88–87.06), and turning rates (573–2905) were significant among experiments ($356.48 < \chi^2 < 2022.09$, $p < 0.02$) (Table 1). The variance in speed tended to increase with fish weight ($p < 0.011$), and the variance in the turning rate tended to increase with water temperature ($p < 0.004$).

Models

Our experiments allowed us to develop relationships between spontaneous swimming costs and fish weight (Table 2). The slope of the allometric model that we obtained ($r^2 = 0.81$) was not significantly different from unity ($p > 0.05$), indicating the presence of a direct proportionality between spontaneous swimming costs and fish weight. Using a linear model, fish weight explained 66% of the variation in spontaneous swimming costs. No descriptor of fish swimming characteristics, alone or combined with fish weight, using an allometric or linear model, explained a significant fraction of variations in spontaneous swimming costs. Dixon's test performed using the residuals of our model indicated that experiment 16 represented an outlier ($p < 0.05$) (Sokal and Rohlf 1995). Exclusion of this experiment resulted in a linear model in which fish weight explained 79% of variations in spontaneous swimming costs (Table 2).

Combination of the data from our 24 experiments with those of Tang and Boisclair (1995) (18 experiments) slightly increased the range of fish weight included in our analysis (from 3.1–41.6 to 1.2–41.6 g) but had no effect on the range of swimming characteristics that we used. The three experiments of Tang and Boisclair (1995) performed at 21°C were excluded from our analysis because such temperatures may negatively affect the behaviour of brook trout and complicate the interpretation of the respiration rates of these fish. The data set of 42 experiments confirmed the existence of a strong relationship between spontaneous swimming costs and fish weight (Table 2). The allometric model that we obtained suggested that fish weight explained 91% of the variation in spontaneous swimming costs (Table 2). The slope of this model (1.13, SD = 0.06) was statistically different from unity ($p < 0.05$).

Discussion

Our analyses indicate that, in certain models, fish weight can explain 91% of the variation in spontaneous swimming costs. Neither water temperature nor fish swimming characteristics contributed to the models, a finding that contrasts with published studies on the cost of spontaneous swimming. For instance, the empirical analysis performed by Boisclair and Tang (1993) suggested that swimming speed could explain 60% of the variation in spontaneous swimming costs for three fish species. Similarly, Krohn and Boisclair (1994) and Tang and Boisclair (1995) reported that characteristics such as swimming speed and average turning rate could explain from 35 to 83% of the variance in spontaneous swimming costs of brook trout. The difference between the structure of the model that we found and that presented in published studies may be related to the range of the independent variables used to develop the models. However, the range of average swimming speed observed in our experiments (3.8–13.5 $cm \cdot s^{-1}$) is similar to that used in the empirical analysis of Boisclair and Tang (1993) (0.4–8.3 $cm \cdot s^{-1}$) and found by Tang and Boisclair (1993) (6.4–11.1 $cm \cdot s^{-1}$) in 1- to 27-m^3 enclosures. Furthermore, our range of speed is even larger than that observed by Krohn and Boisclair (1994) (2.3–6.6 $cm \cdot s^{-1}$) and Tang and Boisclair (1995) (4.0–9.7 $cm \cdot s^{-1}$). In fact, the ranges that we obtained for all swimming characteristics (turning rate, variance in turning rate, variance in speed, variance in acceleration) are larger than those in other studies on the spontaneous swimming costs of brook trout. One key element may be that our study also covers the widest relative range of fish weight published so far to model spontaneous swimming costs. In other studies, fish weight varied 1.2-fold (Tang and Boisclair 1995) to 12.5-fold (Boisclair and Tang 1993). In our study, fish weight varied 38-fold among experiments. With the exception of our own work, the only other study in which fish weight explained a significant fraction (16%) of spontaneous swimming costs was the empirical analysis of Boisclair and Tang (1993), and it is also the data set that contained, on a relative basis, the second most variable range of fish weight (8–100 g wet, 12.5-fold difference). This suggests that the structure of the model that we found is largely determined by the range of the variables that we used.

We compared the allometric model obtained combining our data with those Tang and Boisclair (1995) with the model proposed by Boisclair and Tang (1993). For that purpose, we used fish weight and mean swimming speed values (from this study and those of Tang and Boisclair 1995) as inputs to the model of Boisclair and Tang (1993) and compared the predictions of this model with the predictions of our allometric model (Fig. 1). Predictions produced by the model of Boisclair and Tang (1993) tended to parallel those of our allometric model. However, these predictions differed, on average, by 83.5% (1.6–152%). Two factors may contribute to explaining the numerical difference between these models. First, the empirical model of Boisclair and Tang (1993) for spontaneous swimming was developed for fish ranging from 8 to 100 g wet weight. Hence, some of the predictions described above have been made outside the range appropriate for using this model. Exclusion of predictions for fish weighing less than 8 g decreased the relative difference between both models to an average of 48.6%. Second, the model of Boisclair and Tang (1993) was developed using results from three fish species other than brook trout. In addition, the protocols used to estimate swimming speed (using grids and different visual approaches) were very different for each study. It is currently difficult to assess if a single model can be appropriate for estimating the cost of spontaneous swimming for many fish species or for evaluating the effect of sampling protocols on the predictions made by the models.

Despite structural and numerical differences between the models cited above, our study confirms that the cost of spontaneous swimming, for given fish weight and swimming speed combinations, is larger than that of forced swimming. While forced swimming is defined as a movement in which fish speed and direction are constant (as swimming in a flume), fish in our experiments, as under most natural conditions, could accelerate and turn. The energetic implications of these differences can be quantified by comparing oxygen consumption predicted by our allometric model with predictions made by the forced swimming model developed by Boisclair and Tang (1993). The forced swimming model of Boisclair and Tang (1993) uses fish weight and swimming speed as independent variables and was developed for fish weight ranging from 1.8 to 100 g wet and swimming speed from 0.3 to 20 $cm \cdot s^{-1}$. The comparison between our spontaneous model and the forced model of Boisclair and Tang (1993) was made using values of fish weight and speed obtained during our experiments. Spontaneous swimming costs predicted by our model were 3- to 22-fold larger than predictions made by the forced swimming model (average of 8.2-fold) (Fig. 1). Hence, our observations confirm, with a wider range of experimental data on spontaneous swimming (see Krohn and Boisclair 1994; Tang and Boisclair 1995), that use of forced swimming models may greatly underestimate the cost of swimming for fish that perform changes in speed and direction. This situation may have an equally strong effect on predictions made by many ecological models. Forced swimming models have been used to estimate the energetic value of a habitat for fish (expected energy acquired by fish under given environmental conditions: Feldmeth and Jenkins 1973; Braaten et al. 1997) and the behaviour that fish should adopt to maximize or optimize their energy gain (optimal swimming speed under a given set of environmental conditions: Ware 1975; Dabrowski et al. 1988). Forced swimming models have also been integrated into bioenergetic models and currently represent the standard approach that allows the use of values of fish swimming speed to assess the energy requirements of fish populations (Stewart et al. 1983; Hewett and Johnson 1992; Rand et al. 1993). Considering the magnitude of the difference that we observed between predictions made by forced and spontaneous swimming models, our work suggests that many ecological models that depend on estimates of swimming costs may underestimate total energy expenditures for fish that perform changes in speed and direction. This may result in an overestimation of optimal swimming speed values and in an underestimation of fish consumption rates predicted by bioenergetic models. By extension, an underestimation of fish swimming costs may also cause an overestimation by bioenergetic models of the carrying capacity of aquatic systems and of the fish stocking rate that may be sustainable in these systems.

Acknowledgments

We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fonds pour la formation de Chercheurs et l'Aide à la Recherche (FCAR) for their financial support through grants to D.B. (NSERC research grant, FCAR-Nouveau chercheur) and to the freshwater ecology group of Université de Montréal (FCAR-Équipe). M.T. was supported by a postgraduate fellowship from NSERC, the Fonds spécial de la Faculté des Études Supérieures from Université de Montréal, the Fondation Joseph–Arthur–Paulhus, and a scholarship obtained through the Groupe de Recherche Interuniversitaire en Limnologie (GRIL, FCAR-Centre).

References

Beamish, F.W.H. 1978. Swimming capacity. *In* Fish physiology. Vol. 7. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 101–187.

Beamish, F.W.H., and Dickie, L.M. 1967. Metabolism and biological production in fish. *In* The biological basis of freshwater fish production. *Edited by* S.D. Gerking. Blackwell, Oxford, U.K. pp. 215–242.

Boisclair, D. 1992*a*. An evaluation of the stereocinematographic method to estimate fish swimming speed. Can. J. Fish. Aquat. Sci. **49**: 523–531.

Boisclair, D. 1992*b*. Relationship between feeding and activity rates for actively foraging. juvenile brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **49**: 2566–2573.

Boisclair, D., and Tang, M. 1993. Empirical analysis of the influence of swimming pattern of the net energetic cost of swimming in fishes. J. Fish Biol. **42**: 169–183.

Braaten, P.J., Dey, P.D., and Annear, T.C. 1997. Development and evaluation of bioenergetic-based habitat suitability criteria for trout. Regul. Rivers Res. Manage. **13**: 345–356.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. **21**: 1183–1226.

Brett, J.R., and Glass, N.R. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. **30**: 379–387.

Brett, J.R., and Groves, T.D.D. 1979. Physiology energetics. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 280–357.

Dabrowski, K., Takashima, F., and Law, Y.K. 1988. Bioenergetic model of planktivorous fish feeding, growth, and metabolism: theoretical optimum swimming speed of fish larvae. J. Fish Biol. **32**: 443–458.

Feldmeth, C.R., and Jenkins, T.M. 1973. An estimate of energy expenditure by rainbow trout (*Salmo gairdneri*) in a small mountain stream. J. Fish. Res. Board Can. **30**: 1755–1759.

Fry, F.E.J., and Hart, J.S. 1948. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. (Woods Hole), **94**: 66–77.

Hewett, S.W, and Johnson, B.L. 1992. A generalized bioenergetics model of fish growth for microcomputer. Univ. Wis. Sea Grant Tech. Rep. No. WIS SG-92-250.

Holliday, F.G.T., Tytler, P., and Young, A.H. 1974. Activity levels of trout (*Salmo trutta*) in Airthrey Loch, Stirling, and Loch Leven, Kinross. Proc. R. Soc. Edinburgh Sect. B (Biol.), **74**: 315–331.

Hughes, N.F., and Dill, L.M. 1990. Position choice by drift-feeding salmonids: model and test for Arctic grayling (*Thymallus arcticus*) in a subarctic mountain stream, interior Alaska. Can. J. Fish. Aquat. Sci. **47**: 2039–2048.

Job, S.V. 1955. The oxygen consumption of *Salvelinus fontinalis*. Univ. Toronto Stud. Biol. Ser. No. 61. Publ. Ont. Fish. Res. Lab. No. 73.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*). J. Fish. Res. Board Can. **34**: 1922–1935.

Krohn, M., and Boisclair, D. 1994. The use of a stereo-video system to estimate the energy expenditure of free-swimming fish. Can. J. Fish. Aquat. Sci. **51**: 1119–1127.

Ménard, C. 1991. Utilisation de la vidéo pour mesurer les poissons in situ. Mémoire de maîtrise, Université de Montréal, Montréal, Qué.

Rand, P.S., Stewart, D.J., Seelbach, P.W., Jones, M.L., and Wedge, L.R. 1993. Modeling steelhead population energetics in Lakes Michigan and Ontario. Trans. Am. Fish. Soc. **122**: 997–1001.

Ross, L.G., Watts, W., and Young, A.H. 1981. An ultrasonic biotelemetry system for the continuous monitoring of tail-beat rate from free-swimming fish. J. Fish Biol. **18**: 479–490.

Sirois, P., and Boisclair, D. 1995. The influence of prey biomass on activity and consumption rates of brook trout (*Salvelinus fontinalis* Mitchill). J. Fish Biol. **46**: 787–805.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry. 3rd ed. W.H. Freeman and Company, New York.

Stewart, D.J., Weininger, D., Rottiers, D.V., and Edsall, T.A. 1983. An energetics model for the lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. Can. J. Fish. Aquat. Sci. **40**: 681–698.

Tang, M., and Boisclair, D. 1993. Influence of the size of enclosures on the swimming characteristics of juvenile brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **50**: 1786–1793.

Tang, M., and Boisclair, D. 1995. Relationship between respiration rate of juvenile brook trout (*Salvelinus fontinalis*), water temperature, and swimming characteristics. Can. J. Fish. Aquat. Sci. **52**: 2139–2145.

Ware, D.M. 1975. Growth, metabolism, and optimal swimming speed of a pelagic fish. J. Fish. Res. Board Can. **32**: 33–41.

Weatherley, A.H., Rogers, S.C., Pincock, D.G., and Patch, J.R. 1982. Oxygen consumption of active rainbow trout, *Salmo gairdneri* Richardson, derived from electromyograms obtained by radiotelemetry. J. Fish Biol. **20**: 479–489.

Webb, P.W. 1991. Composition and mechanics of routine swimming of rainbow trout, *Oncorhynchus mykiss*. Can. J. Fish. Aquat. Sci. **48**: 583–590.

Influence of inorganic nitrogen cycling on the δ^{15}N of Lake Ontario biota

M.F. Leggett, O. Johannsson, R. Hesslein, D.G. Dixon, W.D. Taylor, and M.R. Servos

Abstract: The seasonal variability in the δ^{15}N of biota at the base of the pelagic food chain in Lake Ontario was studied during the period 1995–1997. Strong seasonal patterns were observed in the δ^{15}N of dissolved inorganic nitrogen, different size fractions of particulate organic matter, and zooplankton split into size fractions of 110–210, 210–295, and >295 µm. Similar patterns were reflected in the δ^{15}N of individual species: *Diacyclops thomasi*, *Bosmina longirostris*, *Daphnia* spp., and calanoid copepods. The seasonal fluctuation of primary producers was a function of the nitrogen enrichment of dissolved inorganic nitrogen during photosynthetic uptake and the δ^{15}N of NH_4^+ and NO_3^- used by the algae. The large range in δ^{15}N of primary producers suggests that recycling of nitrogen is an important determinant of δ^{15}N at the base of the food web. The δ^{15}N of individual species of zooplankton reflected the known feeding strategies and dietary preferences.

Résumé : On a étudié la variabilité saisonnière de δ^{15}N dans le biote de la base de la chaîne alimentaire pélagique du lac Ontario pendant la période de 1995 à 1997. Des patrons saisonniers importants ont été observés dans le δ^{15}N de l'azote inorganique dissous, de différentes classes de taille de matière organique particulaire et de trois classes de taille de zooplancton (110–210, 210–295 et >295 µm). On a également relevé des patrons semblables dans le δ^{15}N chez certaines espèces, notamment *Diacyclops thomasi*, *Bosmina longirostris*, *Daphnia* spp. et les copépodes calanoïdes. La variation saisonnière des producteurs primaires était fonction de l'enrichissement en azote de l'azote inorganique dissous pendant l'absorption photosynthétique et des valeurs de δ^{15}N dans le NH_4^+ et le NO_3^- utilisé par les algues. La vaste gamme de valeurs de δ^{15}N chez les producteurs primaires laisse supposer que le recyclage de l'azote joue un rôle important dans le δ^{15}N à la base du réseau trophique. Les valeurs de δ^{15}N chez certaines espèces de zooplancton ont confirmé les stratégies et les préférences alimentaires déjà connues.

[Traduit par la Rédaction]

Introduction

The nitrogen isotope signatures (δ^{15}N) of aquatic organisms are often used to indicate relative trophic position in food webs (e.g., Cabana and Rasmussen 1994). In aquatic systems, the δ^{15}N of primary producers and consumers may vary spatially and temporally within and between systems (e.g., Yoshioka et al. 1994; Gu et al. 1996). The δ^{15}N of primary producers is a function of the signature of inorganic or organic nitrogen utilized by algae and the level of isotope discrimination expressed in the process of nutrient uptake (e.g., Goericke et al. 1994). The level of isotope discrimination is related to relative concentrations of dissolved inorganic nitrogen (DIN) as well as species specificity (Goericke et al. 1994; Waser et al. 1998). The DIN varies considerably across freshwater systems and may also fluctuate on a seasonal basis within a system (Hecky and Kilham 1988). Few studies have extensively characterized the base of the food web in a lacustrine system over an entire season or in relation to the nitrogen biogeochemistry of the system (e.g., Owens 1987; Cifuentes et al. 1988). Seasonal changes in biogeochemistry are potentially responsible for the variation in the isotope signatures of primary consumers observed in previous studies of Lake Ontario (Kiriluk et al. 1995). Variability in the δ^{15}N of the forage base will have implications for assessment of relative trophic position of organisms higher in the food web.

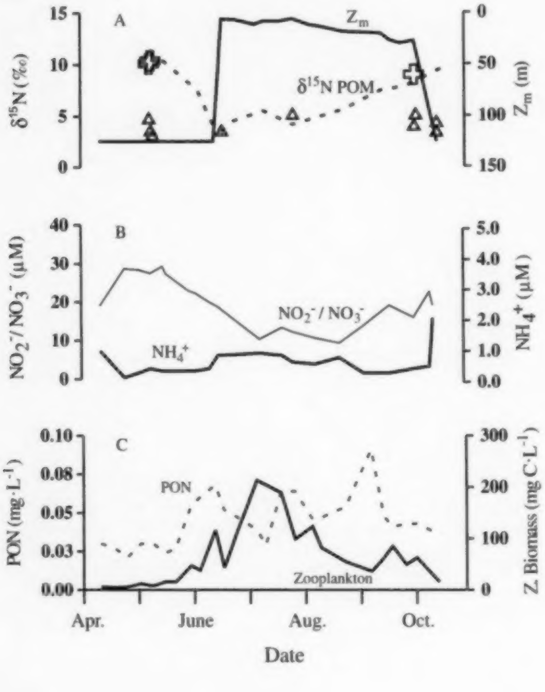
This study was intended to determine the seasonal pattern and level of variability in δ^{15}N at the base of the Lake Ontario food web. The δ^{15}N values of DIN, particulate organic matter (POM), and zooplankton were analyzed seasonally at two locations in the lake. A conceptual model of the flow of nitrogen through the base of the food web was developed.

Received June 2, 1998. Accepted February 10, 2000.
J14629

M.F. Leggett,[1] D.G. Dixon, and W.D. Taylor. Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada.
O. Johannsson. Fisheries and Oceans Canada, Great Lakes Laboratory for Fisheries and Aquatic Sciences, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.
R. Hesslein. Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada.
M.R. Servos. Environment Canada, National Water Research Institute, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

[1] Author to whom all correspondence should be addressed: Cyanamid Crop Protection, 88 McNabb Street, Markham, ON L3R 6E6, Canada.
e-mail: michael_leggett@py.cyanamid.com

Fig. 1. (A) Seasonal change in the $\delta^{15}N$ of POM at Station 41, Lake Ontario, 1995, in relation to the $\delta^{15}N$ of NO_2^-/NO_3^- (△), the $\delta^{15}N$ of NH_4^+ (✚), and the mixing depth (Z_m). (B) Seasonal change in the surface water concentrations of NO_2^-/NO_3^- and NH_4^+ (taken from 1–20 m when the lake was mixed and the midepilimnion when the lake was stratified). (C) Particulate organic nitrogen (PON) concentrations and zooplankton biomass at Station 41, 1995.



Materials and methods

Water, POM, and zooplankton samples were collected biweekly from a midlake station (Station 41) and from a site in the Kingston basin (Station 81) from the beginning of April to the end of October of 1994 and 1995. These sites were part of the Bioindex long-term biomonitoring program (Johannsson et al. 1998). Additional samples for $\delta^{15}N$ analysis of nitrate (NO_3^-) in water were collected April 23, September 24, and October 23, 1997, from Station 41. Samples for NO_3^- analysis were collected July 23, 1997, from Station 403, also in the offshore, 5 km southeast of Station 41.

Ten-litre samples of water, filtered through GF/F filters, were collected in polyethylene containers and stored frozen for $\delta^{15}N$ analysis of NH_4^+ and NO_2^-/NO_3^-. NH_4^+ was extracted from water samples using activated zeolite (Velinsky et al. 1989; H. Bootsma, Fisheries and Oceans Canada, Freshwater Institute, Winnipeg, Man., personal communication). Activated zeolite was added to the sample in a 2-L separatory funnel and shaken for 5 min. The zeolite was then collected from the sample on a precombusted GF/F filter that was dried in a desiccator under vacuum. After all of the NH_4^+ in the sample was removed, the NO_2^-/NO_3^- in the sample was reduced to NH_4^+ by passing the sample through a zinc column activated with 2 N H_2SO_4. The NH_4^+ obtained from NO_2^-/NO_3^- reduction was then extracted with zeolite as described above. The dried zeolite was scraped from the filters into glass vials and stored at −4°C prior to analysis.

The analysis is of the $\delta^{15}N$ of DIN was made difficult by the low ambient concentrations of ammonium (NH_4^+) in the lake on many of the occasions when sampling took place. The $\delta^{15}N$ of NH_4^+ could not be measured at Station 81. The method of analysis was validated for NO_3^- with 30 µM KNO_3 standards of known isotopic composition prior to study initiation. The reduction and extraction efficiency of these standards was >95%. Results were within 1‰ of the known signature. Standards of $(NH_4)_2SO_4$, with known $\delta^{15}N$, were extracted and analyzed on an ongoing basis. On average, there was a difference of −0.65 ± 0.66‰ between these standards and the known $\delta^{15}N$ when the extraction efficiency was >85%. A correction factor of 0.65‰ was added to the $\delta^{15}N$ of the samples analyzed to correct for fractionation in extraction. For many of the samples, the initial concentration of NH_4^+ was low, so that a >90% extraction resulted in a residual NH_4^+ concentration below the 0.005 mg·L^{-1} (0.4 µM) level of detection. The concentrations of NO_2^-/NO_3^- were comparatively high, between 10 and 30 µM NO_2^-/NO_3^-. Therefore, after NO_2^-/NO_3^- reduction, greater extraction efficiencies for NH_4^+ were attainable.

Particulate samples were sequentially filtered through a series of mesh screens 295, 210, 110, and 64 µm in diameter. The effects of alternative methods of sample storage and pretreatment on the $\delta^{15}N$ of zooplankton were investigated. No difference in the $\delta^{15}N$ was observed between lipid-extracted and (or) acid-treated zooplankton, zooplankton stored in ethanol, and zooplankton that were fresh-frozen and analyzed with no other pretreatment. Refer to Leggett (1998) for a detailed description of the methods used in sample preparation.

Carbon and nitrogen isotope ratios for all particulate and zooplankton samples were obtained using a VG Optima continuous-flow isotope ratio mass spectrometer. Instrument accuracy, precision, and range of linearity were monitored using National Institute of Standards and Technology (U.S.A.) standards IAEA-N1 and IAEA-N2. Duplicate standards of identical mass within the linear range of instrument operation consistently had measured $\delta^{15}N$ values within 0.25‰ of each other.

Results

Temporal fluctuations in $\delta^{15}N$ were observed in DIN, all size fractions of POM, and the various species of zooplankton analyzed. Differences were also observed between size fractions of POM collected at the same time and site.

In the spring, the $\delta^{15}N$ of NO_2^-/NO_3^- in the surface water at Station 41 was 3.7‰ in 1995 and 3.25‰ in 1997 (Fig. 1; Table 1). Spring values for NH_4^+ obtained in 1995 were approximately 8‰ heavier than those for NO_2^-/NO_3^- (Fig. 1). The concentration and $\delta^{15}N$ of NO_2^-/NO_3^- measured in 1997 were consistent from the surface to the lake bottom prior to stratification. The concentration of NO_2^-/NO_3^- in the epilimnion decreased from the time of stratification to midsummer. The spring concentration and $\delta^{15}N$ of NO_2^-/NO_3^- at Station 81 were similar to those at Station 41 (Fig. 2).

After the onset of stratification, the concentrations and $\delta^{15}N$ of NO_2^-/NO_3^- at different depths in the water column appeared to diverge (Table 1). The concentration of NO_2^-/NO_3^- declined in the epilimnion but not in the hypolimnion. The $\delta^{15}N$ of NO_2^-/NO_3^- in the hypolimnion appeared to drop relative to signatures obtained in the spring (Table 1). No pattern of increase or decrease was observed in the $\delta^{15}N$ of epilimnetic NO_2^-/NO_3^-. The $\delta^{15}N$ of DIN samples collected at Station 41 was essentially the same in October 1995 and October 1997 at approximately 4‰. No measurements of the $\delta^{15}N$ of NH_4^+ were obtained for the stratified period.

Table 1. $\delta^{15}N$ of NO_2^-/NO_3^- in water samples (*n*) collected at various depths in Lake Ontario in April 1997 prior to stratification and at depths representative of the hypolimnion (75 m) and epilimnion (3 m) after stratification.

Date	Depth (m)	Concentration (mg·L^{-1})	SD	*n*	$\delta^{15}N$ (‰)	SD	*n*
April	3	0.400	0.006	5	3.25		2
	50	0.388		1	3.03		1
	120	0.420	0.015	3	3.06		2
July	3	0.299	0.031	4	4.85	1.840	3
	75	0.546	0.024	3	2.27		2
August	3	0.251			6.06		1
September	3	0.266	0.009	6	5.03	0.950	3
	75	0.450	0.024	3	–1.15	2.010	4
October	3	0.296	0.015	5	3.805	1.470	4
	75	0.435	0.017	3	–1.70		2

Fig. 2. (A) Seasonal change in the $\delta^{15}N$ of POM at Station 81, Lake Ontario, 1995, in relation to the $\delta^{15}N$ of NO_2^-/NO_3^- (△) and the mixing depth (Z_m). (B) Seasonal change in the surface water concentrations of NO_2^-/NO_3^- and NH_4^+ (taken from 1–20 m when the lake is mixed and the midepilimnion when the lake is stratified). (C) Particulate organic nitrogen (PON) concentrations and zooplankton biomass at Station 81, 1995.

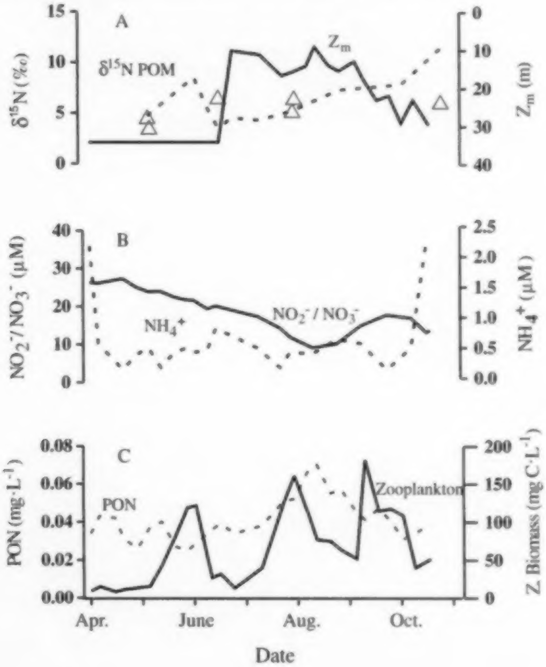
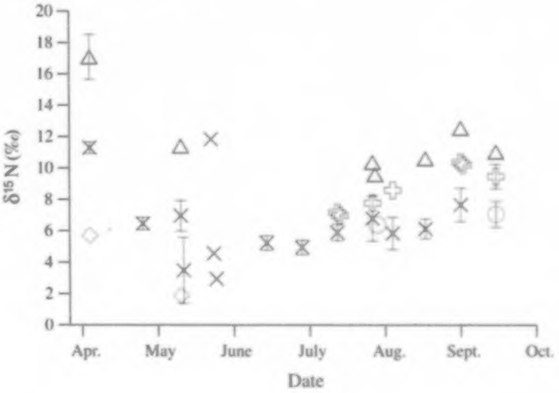


Fig. 3. Seasonal trends in zooplankton and particulate $\delta^{15}N$ at Station 81, Lake Ontario, 1994. ◇, POM 20–64 μm; ×, POM 64–110 μm; ✙, POM 110–210 μm; △, zooplankton 210–295 μm; ○, zooplankton >295 μm.



The $\delta^{15}N$ of particulates collected on GF/F filters (1–20 μm) dropped immediately after the onset of thermal stratification (Figs. 1 and 2). Over the course of the summer, the isotope signature of this particulate fraction increased through to fall mixing at both sites in both 1994 and 1995.

The intermediate size fractions collected (64–110 and 110–210 μm) contained both phytoplankton and small zooplankton. There was a broad range in $\delta^{15}N$ of particulate samples collected at Station 81 in the spring (Fig. 3). Spring samples, determined by microscopic inspection to be "cleaner" (less algal biomass), also had higher and less variable $\delta^{15}N$. From June through September, the particulate samples >64 μm were mainly composed of rotifers. The >64 μm samples, collected August 1 and 17, were each rinsed prior to analysis and contained varying levels of phytoplankton, which shows up as a larger range of standard deviations (Fig. 3). The influence of algae was opposite that in the spring: the lower the percentage of phytoplankton in the sample, the lower the $\delta^{15}N$ of these samples. The >110 μm fraction, composed of rotifers, copepodites, ciliates, and dinoflagellates, was often enriched relative to cladocerans (Fig. 3).

Macrozooplankton populations in the spring consist of cyclopoid copepods, predominantly *Diacyclops thomasi*, and calanoid copepods. The $\delta^{15}N$ of the copepods, collected in the spring prior to stratification at both sites, was approximately 18‰. This was more than 9‰ higher than that of any of the particulates collected in the spring and 2–3‰ higher than the average lake trout (*Salvelinus namaycush*) signatures from Kiriluk et al. (1995). The signatures of copepods dropped substantially (approximately 8.0‰) at the onset of stratification (Tables 2 and 3).

Table 2. $\delta^{15}N$ and number of samples analyzed for individual species of zooplankton collected seasonally at Station 41 in Lake Ontario.

	Diacyclops thomasi		Calanoid copepods		*Bosmina* spp.		*Daphnia* spp.	
Date	$\delta^{15}N$	*n*	$\delta^{15}N$	*n*	$\delta^{15}N$	*n*	$\delta^{15}N$	*n*
1994								
May 12	19.96	1	17.37	1				
May 26	18.19	1	17.37	1				
June 6	14.67	1	17.97	2				
June 30	10.82±0.42	5	10.29	1	4.35	2		
July 14	8.87	1			3.84	2		
July 28	9.72	1			4.69	1		
August 11	9.32	2	5.00	2	5.52	2	4.30	1
August 18	9.32±0.63	3			5.28±0.88	3		
September 27	10.92	2						
October 6	13.01	1	12.27	1	7.53	2	9.21	1
October 13	12.17	1	7.68	2			9.61	1
October 13			13.01	1				
1995								
May 11	19.07	2	18.66	2				
June 16	10.15±0.22	6	13.90	1	5.71	2		
June 21	9.40	1	6.94	1	5.51	2		
June 29					7.86	1	4.83	2
August 1	8.62	2	6.87	1	5.28±0.63	3	9.02	1
September 26	12.92	1	11.55	1	7.50	1	11.56	1
October 23			15.05	1	12.64	1		

Note: Samples are composites picked from zooplankton net hauls taken from 20 m to the surface when the lake was mixed and from the midepilimnion to the surface when the lake was thermally stratified.

Table 3. $\delta^{15}N$ and number of samples analyzed for individual species of zooplankton collected seasonally at Station 81 in Lake Ontario.

	Diacyclops thomasi		Calanoid copepods		*Bosmina* spp.		*Daphnia* spp.	
Date	$\delta^{15}N$	*n*	$\delta^{15}N$	*n*	$\delta^{15}N$	*n*	$\delta^{15}N$	*n*
1994								
May 10								
May 25	15.22	2	14.96	1	11.05	1		
June 7			13.80	2				
June 28	8.17	1	7.55	1	7.21	1		
July 12	4.95	1						
July 12	10.47	1			8.83	1		
July 26	8.58							
August 11	7.16	1						
August 11			10.65	1	9.52	1		
August 30								
September 13					7.39±0.45	3		
September 27			11.77	1				
1995								
May 10	18.03±0.19	5	13.79	2				
June 15			7.94	1				
June 20	10.38±0.25	4	9.31	2	5.60	2		
June 28					4.66	1		
July 14			9.13	1	7.40±0.67	3		
August 2	9.86	2			5.89	1	5.49±0.44	3
September 27	10.94	2	12.18	1	7.53	2	6.94	2
October 20	13.64	2	13.82	1			10.10	1

Note: Samples are composites picked from zooplankton net hauls taken from 20 m to the surface when the lake was mixed and from the midepilimnion to the surface when the lake was thermally stratified.

Cladocerans were first sampled when the lake became stratified and populations were established. *Bosmina longirostris* was first available in sufficient quantity to obtain a sample in mid-June at Station 41. The $\delta^{15}N$ of the cladocerans *B. longirostris* and *Daphnia* spp. was consistently between 0 and 2‰ higher than that of the 1–20 µm size fraction of particulates collected at Station 41 at the same time (Fig. 1). *Daphnia* spp. generally had an isotope composition similar to that of *B. longirostris* at both sites. The $\delta^{15}N$ of *B. longirostris* and *Daphnia* spp. was approximately the same or slightly lower than that of all of the fractions of POM >64 µm collected from June through October in 1994 and 1995 (Fig. 3). Both species showed a moderate seasonal increase in $\delta^{15}N$ from August through October when present that late in thre season (Tables 2 and 3). The $\delta^{15}N$ of *D. thomasi* was consistently 3.0–5.0‰ enriched in ^{15}N relative to *B. longirostris* and *Daphnia* spp. and most of the particulate samples from mid-June through to October.

Discussion

If $\delta^{15}N$ measurements are used as a means of assessing the relative trophic position of different organisms, the baseline of the system should be characterized to account for fluctuations that may occur as the result of changes in the nutrient dynamics and seasonal succession of species within the system. Almost all of the observed seasonal variability in the $\delta^{15}N$ of the zooplankton species analyzed could be attributed to the seasonal fluctuation in the $\delta^{15}N$ of POM. The variability between species and among different size-classes was a function of differences in trophic status and heterogeneity in the $\delta^{15}N$ of source nitrogen at the base of the food web.

Influence of inorganic nitrogen on the $\delta^{15}N$ of POM

The $\delta^{15}N$ values of the DIN sources may vary temporally as they are influenced by the system biogeochemistry. The $\delta^{15}N$ of DIN in the spring will be determined by the conditions occurring in the lake over the winter into the spring. With the onset of stratification, biological processes in the epilimnion play a more predominant role in the nitrogen cycling influencing the isotope signatures of pelagic organisms in Lake Ontario.

The available ammonia in lakes is derived largely from the sediments and through regeneration from algal and zooplankton excretion. Lakewide surveys have shown elevated concentrations of NH_4^+ in nearshore areas, suggesting that inputs from surface runoff play a limited role (Neilson and Stevens 1987). NH_4^+ produced from the sediment is able to diffuse through the entire water column while the lake is mixed. Since the water column remains oxygenated, much of the NH_4^+ produced is oxidized to NO_3^- and NO_2^- (Axler et al. 1981; Lean and Knowles 1987; Rudd et al. 1988). The remainder is available as an inorganic nitrogen source for primary producers over the winter and into the spring.

NH_4^+ produced through sediment diagenesis is likely to have a $\delta^{15}N$ characteristic of the surface sediment from which it is generated. The $\delta^{15}N$ of sediment organic material at these sites in the spring may be estimated from the signatures of benthic invertebrates (Leggett 1998). Adjusting for trophic enrichment, the sediment signature in April or early May would be approximately 9–10‰. The signature of NH_4^+ regenerated from zooplankton might be estimated from the signature of zooplankton by adjusting for fractionation associated with uptake and trophic enrichment. Excreted NH_4^+ would be approximately 8‰ lower than the organism producing it (Checkley and Entzeroth 1985). Given the signatures of copepods in the spring (≥18‰), NH_4^+ from this source would similarly be approximately 9–10‰. The NH_4^+ signatures determined from extractions of water samples collected in the spring were in good agreement with these estimates (Fig. 1).

NO_3^- and NO_2^- are produced through oxidation of regenerated NH_4^+. Concentrations of NO_3^- across the lake basin remain fairly homogeneous in the spring, suggesting little influence on open-lake concentrations from the major tributaries (Neilson and Stevens 1987). The $\delta^{15}N$ of NO_3^- in the surface waters of marine systems has been correlated with the $\delta^{15}N$ of sediments (Francois et al. 1992; Altabet and Francois 1994). The $\delta^{15}N$ of NO_3^- in the spring was much lower than estimated sediment $\delta^{15}N$. This may be a function of the availability and continued production of NH_4^+ serving as a source pool for nitrification and primary production. In laboratory experiments with *Nitrosomonas europaea* at 30°C, it was determined that there is an isotope enrichment factor of –35‰ associated with nitrification (Mariotti et al. 1981). In any reaction, if the substrate pool is turned over completely in a unidirectional process, no fractionation of isotopes will be evident. Since the pool of available NH_4^+ is consistently being replenished and is used by multiple sources, there is an opportunity for fractionation to occur, producing NO_3^- with a lower $\delta^{15}N$ than NH_4^+ and much of the particulates, early in the seasonal cycle.

The $\delta^{15}N$ of algae using NO_2^-/NO_3^- as a source of nitrogen in Lake Ontario would be expected to have a $\delta^{15}N$ similar to or less than the $\delta^{15}N$ of NO_2^-/NO_3^- (Montoya and McCarthy 1995; Waser et al. 1998). When the lake is mixed, the $\delta^{15}N$ of particulates collected on GF/F filters (<20 µm) is much higher than the measured $\delta^{15}N$ of NO_2^-/NO_3^- and is closer to the measured/estimated $\delta^{15}N$ of NH_4^+ (Fig. 1). This suggests that NH_4^+, and not NO_2^-/NO_3^-, is the inorganic source of nitrogen for the majority of the material collected on this filter in the spring. Larger size fractions collected had lower $\delta^{15}N$ values, suggesting a difference between species in either the level of fractionation observed and (or) the source of nitrogen used. This finding is consistent with the results of isotope addition experiments conducted during a previous assessment of nitrogen cycling in Lake Ontario (Lean and Knowles 1987). ^{15}N tracer studies conducted from April to June 1982 indicated that smaller cells, <12 µm, were responsible for most of the NH_4^+ uptake, while larger cells used mostly NO_3^- (Lean and Knowles 1987). The POM collected on GF/F filters (1–20 µm) in May was likely to be made up of cryptophytes, dinoflagellates, and chrysophytes, since the species represented within these classes are typically <20 µm in diameter (species composition provided by O. Johannsson, unpublished data). Larger size fractions contained greater proportions of *Melosira* sp. A similar broad range in $\delta^{15}N$ values for different particulate size fractions was observed in studies of eutrophic Lake Suwa, Japan

(Yoshioka et al. 1994). In Lake Suwa, as in Lake Ontario, the wide range in $\delta^{15}N$ between different fractions of POM was due to variation in the species composition of each sample. Netplankton samples (horizontal tows at 1 m depth with a 153-µm-mesh net) collected from Lake Ontario in May 1992 had a $\delta^{15}N$ of 1.7‰ (Kiriluk et al. 1995). The collected material would consist mainly of diatoms, which would result in the low value for $\delta^{15}N$.

At the onset of stratification, there is a sharp decline in the $\delta^{15}N$ of POM of 1–20 µm (Figs. 1 and 2). The phytoplankton species composition of this POM changed with the onset of stratification, and the observed shift in $\delta^{15}N$ may be a reflection of this change. There may be differences in the source of nitrogen used by primary producers or in the level of isotope fractionation by the algal species present. The carbon to nitrogen ratios and observation confirm that the samples were composed of fresh algal biomass (Leggett et al. 1999).

The spring–summer drawdown of NO_3^- is expected to establish the isotope signature of particulates and drive the $\delta^{15}N$ of NO_3^- to higher levels, given the fractionation associated with NO_3^- uptake by many algal species (Waser et al. 1998). The $\delta^{15}N$ of primary producers drawing on an increasingly enriched pool of NO_3^- would subsequently be expected to show a pattern of seasonal enrichment. Consistent enrichment in the $\delta^{15}N$ of POM (1–20 µm) with a subsequent steady decrease in the concentration of NO_2^-/NO_3^- from the time of stratification to early fall is especially apparent at Station 81 (Fig. 2). However, the observed change in the $\delta^{15}N$ of the NO_2^-/NO_3^- pool is not as great as might be expected from early summer to fall. In this study, no statistically significant trend was detected, and the measured $\delta^{15}N$ of NO_2^-/NO_3^- was not consistently enriched relative to the $\delta^{15}N$ of algae collected at or near the same time. This suggests a low level of fractionation associated with photosynthetic uptake or mediation of the isotope enrichment effect of photosynthetic uptake by nitrification. The observed increase in $\delta^{15}N$ of POM (1–20 µm) while the lake was stratified may have been partially due to a more enriched source of nitrogen than NO_2^-/NO_3^- being used by primary producers.

The heterogeneity in $\delta^{15}N$ between the size fractions of particulates collected while the lake was stratified may also be due to alternative sources of nitrogen with distinct $\delta^{15}N$ being used by coexisting species of algae. Thus, the relative proportion of mixotrophic algae may be reflected in the $\delta^{15}N$ of the particulate samples obtained. Mixotrophic algae will feed on bacteria or ciliates ingesting bacteria, which are assimilating organic nitrogen. The $\delta^{15}N$ of these species is likely to be enriched relative to bacteria or organic nitrogen sources. The $\delta^{15}N$ values of 0.2–1 µm (bacteria) and 0.2 µm – 1000 dalton fractions (organic sources of dissolved nitrogen) were analyzed by Leggett (1998) and found to be slightly enriched relative to the particulates collected on GF/F filters at the same time. The difference between the $\delta^{15}N$ of NO_2^-/NO_3^- and the $\delta^{15}N$ of particulate collected while the lake is stratified may therefore be indicative of the extent to which organic nitrogen is actively recycled in the Lake Ontario pelagic food chain through microbial activity.

In previous studies, concentrations of NO_2^- /NO_3^- have been observed to increase from the onset of stratification near the sediments in Lake Ontario (Lean and Knowles 1987). A fall gradient in NO_3^- concentration develops from east to west due to spatial differences in hypolimnetic enrichment (Neilson and Stevens 1987). The increase in NO_2^- /NO_3^- has been suggested as evidence that nitrification is responsible for stabilizing NH_4^+ concentrations below the euphotic zone (Lean and Knowles 1987; Rudd et al. 1988). There was little increase in the concentration of hypolimnetic NO_2^-/NO_3^- observed in this study; however, in the few samples collected, the $\delta^{15}N$ of NO_2^-/NO_3^- appeared to undergo a 4‰ shift to lower values (Table 1). This observed reduction in the $\delta^{15}N$ of NO_3^- might be a function of fractionation during nitrification. More research should be done to determine if this is the case and if this is generally observed in lacustrine systems.

Fall mixing of the water column makes available hypolimnetic sources of NH_4^+ and NO_2^-/NO_3^- to primary producers. The resulting equilibrium $\delta^{15}N$ of the DIN pools midlake would be expected to more closely approximate the $\delta^{15}N$ of the DIN pools in the hypolimnion while the lake is stratified, due to the relative size of the hypolimnion and the surface layers. Fall mixing should "reset" the $\delta^{15}N$ of NO_2^-/NO_3^- to lower, less-enriched values on a seasonal basis.

Dietary sources determining the $\delta^{15}N$ of zooplankton

The dietary preferences of individual species of zooplankton were reflected in their $\delta^{15}N$. Seasonal patterns in the $\delta^{15}N$ of different species could be traced to seasonal changes in the $\delta^{15}N$ of algal food sources.

Copepods are omnivores, feeding on a mixture of algae and small organisms. This feeding strategy resulted in a high level of nitrogen isotope enrichment in the spring. The $\delta^{15}N$ values of *D. thomasi* and calanoid copepods from Lake Ontario were 17–19‰ (Tables 2 and 3). This is similar to the $\delta^{15}N$ of lake trout in Lake Ontario (Kiriluk et al. 1995). A survey of available literature suggests that a trophic enrichment of 2–3‰ in the $\delta^{15}N$ of zooplankton is expected (Kling and Fry 1992; Graham 1997). Thus, in order for the copepods to become so enriched in ^{15}N, they must be selectively feeding on material within the system that has a $\delta^{15}N$ of approximately 15–16‰. This suggests that copepods (17–19‰) cannot be feeding on diatoms (approximately 2‰) significantly at this time of year. It also suggests that copepods are not feeding exclusively on other primary producers, since the $\delta^{15}N$ of nitrogen available to algae would have to be 4–5‰ heavier than that measured. There is little evidence to support a stepwise trophic enrichment of copepods because no fraction analyzed had a $\delta^{15}N$ between that of copepods and that of the particulate samples. It is likely that the elevated signatures of copepods are due to a combination of trophic enrichment, enriched DIN, and the recycling/enrichment of nitrogen through mixotrophic algae or the microbial loop. It is reasonable to assume that a portion of the particulate biomass in the samples collected in the spring was composed of diatoms with a $\delta^{15}N$ of approximately 2‰. It follows that the rest of the collected material has a $\delta^{15}N$ greater than the signatures measured. The 20–64 µm fraction, collected in April of 1997, had a $\delta^{15}N$ of 6.35‰ (Table 3). If 70% of the sample was composed of algae with a $\delta^{15}N$ of approximately 2‰, the remaining 30% would have a $\delta^{15}N$ of approximately 16.5‰. This 30% would consist of mixotrophic algae, ciliates, and rotifers. Mixotrophic algae potentially feed on other phytoplankton, bacteria, protozoans, and ciliates. Assimilation of dissolved organic nitrogen by bacteria and their subsequent ingestion by ciliates and (or) mixotrophs may represent a complex pathway of nitrogen isotope enrichment for copepods feeding on these prey.

When the lake becomes stratified, there is a sharp decline in the $\delta^{15}N$ of POM (1–20 μm) that is reflected in the signature of calanoid and cyclopoid copepods collected at the same time. The difference between the $\delta^{15}N$ of copepods and the POM is also somewhat reduced during the summer. This could indicate a more direct food chain or increased dependence on dominant species in the POM.

The 110–210 and 64–110 μm fractions was generally enriched relative to other particulate samples as well as some of the cladoceran samples. These fractions were dominated by copepodids, rotifers, ciliates, and mixotrophic dinoflagellates, components of the microbial food web. The observed enrichment suggests that at least some of these organisms may be feeding at a higher trophic level or are feeding on an enriched source of POM relative to the cladocerans. This is consistent with the theory of multiple trophic steps within the microbial food web. More detailed characterization of the intermediate size fractions, coupled with isotope and (or) other tracer analysis, may provide some insight into the cycling and recycling of nutrients by smaller organisms in this and other lacustrine systems.

The $\delta^{15}N$ of *D. thomasi* from mid-June through to October is indicative of planktivory and (or) feeding on algae using a ^{15}N-enriched food source (Tables 2 and 3). Feeding experiments indicate that the diet of *D. thomasi* in Lake Ontario is a function of both planktivory on small copepodids, cladocerans, rotifers, ciliates, and rotifer eggs and consumption of mixotrophic algae (Lane 1979; Williamson 1983; LeBlanc et al. 1997).

The lack of observed enrichment in the ^{15}N of cladocerans, relative to POM, suggests that they are feeding selectively on a component of the POM that has a lower $\delta^{15}N$ than the rest of the POM in the sample collected. Calanoid copepods are consistently 2‰ or more enriched, relative to POM, and often have a $\delta^{15}N$ substantially higher than cladocerans collected at the same time (Tables 2 and 3). The observed differences between calanoid copepods and *B. longirostris* may be attributed to a variation in food source or to a difference in trophic status. Calanoid copepods may be feeding on mixotrophic algae or ciliates that are likely to be more ^{15}N enriched for reasons previously discussed.

The level of variability observed is not unique to this system (e.g.,Yoshioka et al. 1994). To avoid the potential for error in assessing relative trophic position, sampling of organisms for isotope analysis should be on a temporal scale corresponding to their potential for tissue turnover and isotopic change. Less frequent sampling should be necessary for fish or other organisms integrating nitrogen on a broader time scale. However, consideration should be given to spatial variation in the isotope signatures of potential food sources and the foraging range of the species whose trophic status is being assessed (Leggett 1998). Better characterization of the seasonal variability of $\delta^{15}N$ at the base of the food web in any given system will provide a better foundation for the interpretation of trophic dynamics on the basis of $\delta^{15}N$ and a better foundation for intersystem trophic comparisons.

Acknowledgments

We wish to thank Donna Graham, Pete Cott, Bob Hess, Debra Myles, and the crews of the CSS *Lauzier* and CSS *Limnos* for their assistance in the collection and preparation of the samples described. We would also like to thank Richard Elgood, Sherry Schiff, and Kent Burnison for their assistance in filtering the water samples. Bill Mark, Bob Drimmie, and the staff of the University of Waterloo Isotope Laboratory provided assistance and expertise in analysis. The comments of Sebastien Lamontagne and two anonymous reviewers on an earlier version of this manuscript helped to shape it into its current form and were much appreciated.

References

Altabet, M.A., and Francois, R. 1994. Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. Global Biogeochem. Cycles, **8**: 103–116.

Axler, R.P., Redfield, G.W., and Goldman, C.R. 1981. The importance of regenerated nitrogen to the phytoplankton productivity in a subalpine lake. Ecology, **62**: 345–354.

Cabana, G., and Rasmussen, J.B. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature (Lond.), **372**: 255–257.

Checkley, D.M., Jr., and Entzeroth, L.C. 1985. Elemental and isotope fractionation of carbon and nitrogen by marine, planktonic, copepods and implications to the marine nitrogen cycle. J. Plankton Res. **7**: 553–568.

Cifuentes, L.A., Sharp, J.H., and Fogel, M.L. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnol. Oceanogr. **33**: 1102–1115.

Francois, R., Altabet, M.A., and Burckle, L.H. 1992. Glacial to interglacial changes in surface nitrate utilization in the Indian sector of the Southern Ocean as recorded by sediment $\delta^{15}N$. Paleoceanography, **7**: 589–606.

Goericke, R., Montoya, J.P., and Fry, B. 1994. Physiology of isotope fractionation in algae and cyanobacteria. *In* Stable isotopes in ecology. *Edited by* K. Lajtha and B. Michener. Blackwell Scientific Publications, Cambridge, Mass. pp. 187–221.

Graham, M. 1997. Influence of omnivory and selective feeding on $\delta^{15}N$-determined trophic position of zooplankton along a gradient of lake production. MSc. thesis, University of Regina, Regina, Sask.

Gu, B., Schelske, C.L., and Brenner, M. 1996. Relationship between sediment and plankton isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and primary productivity in Florida lakes. Can. J. Fish. Aquat. Sci. **53**: 875–883.

Hecky, R.E., and Kilham, P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnol. Oceanogr. **33**: 796–822.

Johannsson, O.E., Dermott, R.M., Feldkamp, R., and Moore, J.E. 1998. The changing pelagia Lake Ontario (1981 to 1995): a report of the DFO long-term biomonitoring (Bioindex) program. Can. Tech. Rep. Fish. Aquat. Sci. No. 2243.

Kiriluk, R.M., Servos, M.R.,Whittle, D.M., and Rasmussen, J.B. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, Mirex, and PCB in a Lake Ontario pelagic food web. Can. J. Fish. Aquat. Sci. **52**: 2660–2674.

Kling, G.W., and Fry, B. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology, **73**: 561–566.

Lane, P.A. 1979. Vertebrate and invertebrate predation intensity on freshwater zooplankton communities. Nature (Lond.), **280**: 391–392.

Lean, D.R.S., and Knowles, R. 1987. Nitrogen transformations in Lake Ontario. Can. J. Fish. Aquat. Sci. **44**: 2133–2143.

LeBlanc, J.S., Taylor, W.D., and Johannsson, O.E. 1997. The feeding ecology of the cyclopoid copepod *Diacyclops thomasi* in Lake Ontario. J. Gt. Lakes Res. **23**: 369–381.

Leggett, M.F. 1998. Food web dynamics of Lake Ontario as determined by carbon and nitrogen stable isotope analysis. Ph.D. thesis, University of Waterloo, Waterloo, Ont.

Leggett, M.F., Servos, M.R., Hesslein, R., Johannsson, O., Millard, E.S., and Dixon, D.G. 1999. Biogeochemical influences on the carbon isotope signatures of Lake Ontario biota. Can. J. Fish. Aquat. Sci. **56**: 2211–2218.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., and Letolle, R. 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles: illustration for the denitrification and nitrification processes. Plant Soil, **62**: 413–430.

Montoya, J.P., and McCarthy, J.J. 1995. Isotopic fractionation during nitrate uptake by phytoplankton grown in continuous culture. J. Plankton Res. **17**: 439–464.

Neilson, M.A., and Stevens, R.J.J. 1987. Spatial heterogeneity of nutrients and organic matter in Lake Ontario. Can. J. Fish. Aquat. Sci. **44**: 2192–2203.

Owens, N.J.P. 1987. Natural variations in ^{15}N in the marine environment. Adv. Mar. Biol. **24**: 389–451.

Rudd, J.W.M., Kelly, C.A., Schindler, D.W., and Turner, M.A. 1988. Disruption of the nitrogen cycle in acidified lakes. Science (Washington, D.C.), **240**: 1515–1517.

Velinsky, D.J., Pennock, J.R., Sharp, J.H., Cifuentes, L.A., and Fogel, M.L. 1989. Determination of the isotopic composition of ammonium-nitrogen at the natural abundance level from estuarine waters. Mar. Chem. **26**: 351–361.

Waser, N.A.D., Harrison, P.J., Nielson, B., Calvert, S.E., and Turpin, D.H. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. Limnol. Oceanogr. **43**: 215–224.

Williamson, C.E. 1983. Invertebrate predation on planktonic rotifers. Hydrobiologia, **104**: 385–396.

Yoshioka, T., Wada, E., and Hayashi, H. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. Ecology, **75**: 835–846.

Evaluating spatially explicit metrics of stream energy gradients using hydrodynamic model simulations

David W. Crowder and Panayiotis Diplas

Abstract: Localized energy gradients and velocity shelters created by boulders, bars, and channel banks are often essential components of aquatic habitat. Two-dimensional hydraulic models have the potential to predict the amounts and locations of such spatially varying flow patterns. However, little effort has been devoted to reproducing these flow features and developing spatial habitat metrics to describe and differentiate between various types of flow patterns. Two-dimensional numerical simulations, based on actual channel geometry, are used here to model a variety of flow patterns encountered in natural streams. The simulation results are used to develop spatial habitat metrics that quantify local velocity gradients and changes in kinetic energy. The proposed metrics are evaluated at various points within the different flow patterns of interest. The metrics produce large values for flow patterns exhibiting considerable spatial variation and small values in areas experiencing uniform flow conditions. Comparisons with other researchers' field data suggest that the metric values produced in the modeled flows are consistent with values found near fish resting and feeding locations. The habitat metrics, measures of the flow's rate of spatial change in kinetic energy, can also be incorporated into bioenergetic models to facilitate the computation of fish energy expenditure rates.

Résumé : Les gradients localisés d'énergie et les abris contre la vitesse du courant créés par les rochers, les barres et les bancs des chenaux sont souvent des composantes essentielles de l'habitat aquatique. Les modèles hydrauliques bidimensionnels ont le potentiel de prédire l'ampleur et la localisation de tels modes d'écoulement à variation spatiale. Toutefois, on a consacré peu de recherche à la reproduction de ces caractéristiques d'écoulement et à l'élaboration d'une métrologie spatiale de l'habitat pour décrire et différencier divers types de régimes d'écoulement. Des simulations numériques bidimensionnelles, basées sur la géométrie réelle du chenal, nous servent ici à modéliser une gamme de régimes d'écoulement observés dans les cours d'eau naturels. Les résultats de la simulation servent à développer une métrologie spatiale de l'habitat qui quantifie les gradients locaux de vitesse et les changements dans l'énergie cinétique. La métrologie proposée est évaluée à différents points entre les différents régimes d'écoulement étudiés. Elle produit des valeurs élevées pour les régimes d'écoulement présentant une variation spatiale considérable, et des valeurs faibles dans les endroits présentant des conditions uniformes d'écoulement. La comparaison avec les données recueillies sur le terrain par d'autres chercheurs permet de penser que les valeurs obtenues dans les écoulements modélisés correspondent aux valeurs observées près des lieux de repos et d'alimentation des poissons. On peut aussi intégrer la métrologie de l'habitat, c'est-à-dire les mesures du taux de changement spatial de l'énergie cinétique des écoulements, aux modèles bioénergétiques, ce qui va faciliter le calcul de la dépense énergétique des poissons.

[Traduit par la Rédaction]

Introduction

Focal point velocity (assumed to be equal to the depth-averaged velocity unless noted otherwise) and depth, along with other parameters, measured at observed fish locations are routinely used to derive habitat suitability criteria. If a location within a stream has parameters that fall within the ranges in which fish were observed to inhabit, the location is deemed suitable habitat. Focal point data alone contain no information on flow direction or on any nearby spatial variations such as eddies, velocity gradients, and transverse flows surrounding the fish. A critical implicit assumption in many habitat studies is that habitat conditions found at a fish's location determine habitat suitability and that flow conditions surrounding the fish are irrelevant.

The assumption that only focal point values influence habitat suitability is consistent with the use of one-dimensional hydraulic models to predict depth and velocity in habitat studies. One-dimensional hydraulic models, such as that used in PHABSIM (physical habitat simulation system), often analyze a river by breaking it into elongated discrete cells, each assumed to have a uniform depth and velocity (Bovee 1978; Milhous et al. 1989). One-dimensional models also assume that the flow in an individual cell does not interact with adjacent cells and that the flow is always in the downstream direction. Consequently, the uniform flow conditions predicted in each cell can easily be compared with focal point depth and velocity values. As the assumptions in one-dimensional models preclude the

Received October 8, 1999. Accepted April 5, 2000.
J15393

D.W. Crowder and P. Diplas.[1] Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, 200 Patton Hall, Blacksburg, VA 24061, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: pdiplas@vt.edu

modeling of two-dimensional features such as eddies, velocity gradients, and transverse flows, additional data describing the flows surrounding a fish's location are of little value.

Some researchers have indicated that spatially varying flow patterns are important to a stream's ecological health. Topographic features (channel shape, boulders, bars, and irregularities along stream banks) create velocity gradients, velocity shelters, wakes, and other mesoscale flow patterns that are important or preferred features of fish, periphyton, and invertebrate habitat (e.g., Lancaster and Hildrew 1993; Hayes and Jowett 1994; Rempel et al. 1999). Disturbed flows (e.g., wakes) downstream of boulders and other objects are used by trout as feeding stations and influence the abundance and diversity of flora and fauna in a stream after a spate (Hayes and Jowett 1994; Biggs et al. 1997). The absence of these topographic features often provides poor stream habitat, as evidenced by the many urban and channelized streams that have simple channel shapes and few spatially varying flow patterns.

The accurate quantification and prediction of amounts and locations of the spatially varying habitats created by mesoscale topographic features is not straightforward. If the traditional approach of collecting habitat data at observed fish locations to determine habitat suitability criteria and then using a hydrodynamic model to help quantify the quality and quantity of stream habitat is to be used, two conditions need to be met. First, the habitat data collected should be capable of quantifying any important spatial variations surrounding the aquatic organisms being studied. Second, the hydrodynamic model chosen to predict the flow field must be able to reproduce these spatial variations.

Several studies have been performed to ascertain the benefits of using two-dimensional hydraulic models to predict depth and velocity values in stream habitat studies (e.g., Leclerc et al. 1995; Ghanem et al. 1996; Tarbet and Hardy 1996). Unlike one-dimensional models, two-dimensional models predict flow velocity in both the downstream and lateral directions. Two-dimensional models also represent the flow as a continuous field instead of independent cells. Hence, two-dimensional models are capable of modeling flow features such as velocity gradients and transverse flows. To date, most two-dimensional model studies have focused on modeling longer reaches of river at rather low resolution. In these lower resolution studies, bathymetry data on mesoscale topographic features (large boulders, root wads, etc.) are typically not incorporated into the model's bathymetry data but are viewed as channel roughness. Without bathymetry data on these features, velocity gradients, velocity shelters, transverse flows, and other spatially varying flow features created by these objects cannot be modeled. Moreover, most two-dimensional model studies have adopted habitat suitability criteria based on focal point data and not spatial habitat metrics that can better quantify and describe the spatially explicit output provided by detailed two-dimensional models.

The two-dimensional simulations used here to evaluate the proposed habitat metrics were specifically designed to model a short reach at high resolution. Extensive efforts were taken in collecting bathymetry data on mesoscale topographic features and developing a finite element mesh capable of modeling the velocity gradients and complex flow patterns created by the mesoscale topography (Crowder and Diplas 2000). The influence that mesoscale topography has on predicted model output, the computational effort needed to reproduce local velocity gradients, and model accuracy, which are topics on their own, are extensively discussed in Crowder and Diplas (2000). Here, only enough information regarding the influence that topographic features have on local flow patterns is given to provide a context of the gradients and degree of influence that the mesoscale topographic features have on flow patterns within the modeled stream reach.

A concerted effort is made here toward developing a general and meaningful way of using and interpreting the spatially explicit output provided by two-dimensional models to better describe and quantify stream habitat. Simple spatial habitat metrics that incorporate local velocity gradients are developed to distinguish between two areas that have similar velocity values but are surrounded by different flow patterns. The parameters are indicative of spatial changes in the flow's kinetic energy and directly relate to fish energy expenditure rates and the proximity to flows that provide velocity shelters or potential high drift rates. Ranges of metric values found at observed fish resting and feeding locations are computed from other researchers' data. The parameter values computed at fish locations are compared with the metric values obtained in the model simulations.

Method of analysis

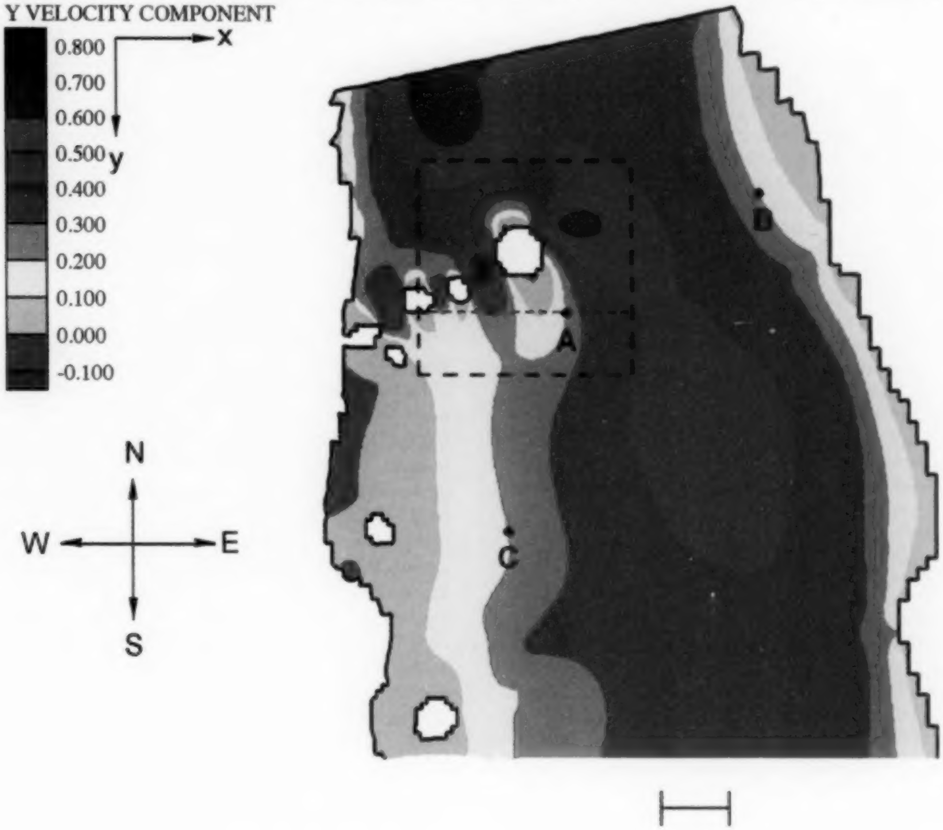
Two-dimensional model development and comparisons

The flow through a 61-m-long pool of the North Fork of the Feather River near Belden, California, U.S.A., was modeled using the two-dimensional finite element computer program RMA-2V (King 1990). The modeled river is a mountainous trout stream that has an average width of 15 m and a maximum depth of approximately 1.5 m. Regulated by an upstream reservoir, the summer time discharge is approximately 4.25 $m^3 \cdot s^{-1}$. Four large boulders are located near the head of the pool. These boulders create a significant velocity shelter and complex localized flow patterns immediately downstream of their position. The presence of these boulders shifts the main current from the west bank of the river to the east bank. Figure 1 illustrates the upper 25 m of the pool. It includes the boulders and the two-dimensional flow patterns created by them.

The study site was modeled three different ways. In each case, the model simulations assumed steady flow. Model output consisted of depth and depth-averaged velocity in the x and y directions at each node point within the finite element mesh. The first simulation modeled the river without bathymetry information on the four exposed boulders at the head of the pool and four more boulders located elsewhere in the pool. The second simulation incorporated bathymetry data on these boulders. The eight boulders had basal areas ranging from 0.56 to 4.10 m^2 and heights from 1.0 to 2.0 m. The third simulation modeled the study site incorporating bathymetry data only on the single largest boulder (one of the four boulders at the head of the pool). Each simulation, based on different bathymetry models, created flow patterns of different complexities.

The flow patterns generated by these numerical simulations are used to represent different spatially varying flows that one might find in natural streams. Computing the values of the proposed spatial habitat metrics at points within these flow patterns allows one to determine if the metric reasonably quantifies and distinguishes between certain flow patterns found in natural rivers. Specifically, these metrics are computed for each simulation along a cross section located immediately downstream of the four boulders at the

Fig. 1. The y velocity components for the upper 25 m of the study site when all boulders are incorporated into the model. The white spots represent the dry portions of the boulders. Color contours provide the magnitude of the y velocity component. The square represents a 6 × 6 m area in which local velocity and kinetic energy per unit mass values were analyzed. The line passing through point A represents the cross section along which velocity, velocity gradient, $v_{\mathrm{ave}} \times |(v_2 - v_1)/\Delta s|$, and $2v_{\mathrm{ave}} \times |(v_2 - v_1)/\Delta s|/v_{\min}^2$ are plotted in Fig. 4. Points A, B, and C are locations that have the same velocity but are surrounded by different velocity gradients. Channel width is 15 m.



Scale: 1 cm = 1.875 m

head of the pool (see Fig. 1). In each model simulation, the cross section runs through an area that has a distinctly different flow pattern. A comparison of the spatial habitat metric values along the cross section is made to determine if the obtained values are capable of distinguishing between flow regions that have fairly uniform velocity profiles from regions that have nonuniform velocity profiles. The ability of the proposed metrics to differentiate between areas having identical focal velocity (0.20 m·s^{-1}), but having different flow features surrounding them, is tested. Specifically, the spatial metric values at three points (points A, B, and C in Fig. 1) having the same velocity, but located in different flow patterns, are compared. While the velocity value of 0.20 m·s^{-1} was chosen arbitrarily, this velocity closely corresponds to the velocity values at brook trout (*Salvelinus fontinalis*) feeding locations observed by Fausch and White (1981). Therefore, points A, B, and C can be viewed as three possible brook trout feeding locations with potentially different metric values. Point A is located in the immediate vicinity of a large boulder that creates significant velocity gradients. Point B is located along the east bank near the transverse flow created by the boulders. Point C is located in the velocity refuge immediately downstream of the four boulders at the head of the pool.

Choice of spatial habitat metrics

A fundamental component of a spatially varying flow pattern is the velocity gradient. Velocity gradients are present near banks, boulders, and other obstructions. Therefore, the velocity gradient is a potentially useful measure for quantifying and distinguishing between flows having similar depth and velocity values but surrounded by different spatially varying flow patterns. Two parameters that incorporate local velocity gradients were evaluated as potential habitat metrics:

$$\frac{\partial}{\partial s}\frac{V^2}{2} = V\frac{\partial V}{\partial s} \cong V_{\mathrm{ave}}\frac{V_2 - V_1}{\Delta s}$$

$$\frac{\dfrac{\partial}{\partial s}\dfrac{V^2}{2}}{\dfrac{V^2}{2}} \cong \frac{\dfrac{1}{2}\left(\dfrac{V_2^2 - V_1^2}{\Delta s}\right)}{\dfrac{V_{\min}^2}{2}} = \frac{2V_{\mathrm{ave}}\dfrac{V_2 - V_1}{\Delta s}}{V_{\min}^2}$$

where V is the magnitude of the depth-averaged velocity, V_1 and V_2 are velocity magnitudes measured a distance Δs apart, V_{ave} is the average of V_1 and V_2, $V_{\min}$ is the minimum value of V_1 and V_2, and

s indicates the direction of the line between points 1 and 2. The first parameter represents the spatial change in a flow's kinetic energy per unit mass and unit length (joules per kilogram per metre). The second parameter divides this spatial change by the kinetic energy of the flow at the point having the lower velocity magnitude. Scaling the spatial change in kinetic energy by the kinetic energy at the point with the smaller velocity provides a measure of how much more energy an organism must expend if it moves from the lower velocity to the higher velocity.

The primary reason for choosing the first parameter is that kinetic energy per unit mass ($V^2/2$) multiplied by a drag coefficient and a frontal area provides the drag force acting on a specific object or organism (Munson et al. 1990). Using the drag force, one can estimate the power expended by a particular organism moving from one location to another. Hence, the parameter has the potential to be employed in individual-based instream flow models (e.g., Rader and Poff 1999) that incorporate an organism's location and energy expenditures into habitat suitability criteria.

Because flow conditions both at the location of the fish and within the surrounding area seem to influence habitat conditions, the second metric might be of more general usefulness. Furthermore, because the difference in energy is scaled by the energy value at the point having the slower velocity, this metric might unify flow conditions at different stream locations, such as banks and boulders, which are frequented by different species or by the same species but at different life stages.

The parameters $V \times \partial V/\partial s$ and $2 \times \partial V/\partial s/V$ apply only at a point in the flow field and may not be accurately computed if Δs is large. If Δs is large, then the use of equations $V_{\mathrm{ave}} \times (V_2 - V_1)/\Delta s$ and $2V_{\mathrm{ave}} \times (V_2 - V_1)/\Delta s/V_{\mathrm{min}}^2$ can be used to accurately calculate the average energy gradient and the average rate that the kinetic energy changes between points 1 and 2 relative to the kinetic energy at the point with the smaller velocity. As the Δs distances used in this paper are relatively large, the latter two expressions are used to compute the spatial habitat parameter values. Selection of an appropriate Δs is discussed later. The potential usefulness of evaluating velocity gradients in different directions or using the x (lateral) or y (downstream) velocity components (u and v) instead of the velocity magnitude ($V = \sqrt{u^2 + v^2}$) in the two proposed parameters is also discussed later. Here, the parameters $v_{\mathrm{ave}} \times |(v_2 - v_1)/\Delta s|$ and $2v_{\mathrm{ave}} \times |(v_2 - v_1)/\Delta s|/v_{\mathrm{min}}^2$ are initially used to evaluate flow conditions in the model simulations. However, the parameters $V_{\mathrm{ave}} \times |(V_2 - V_1)/\Delta s|$ and $2V_{\mathrm{ave}} \times |(V_2 - V_1)/\Delta s|/v_{\mathrm{min}}^2$ were computed for the three model scenarios and are used for comparing the habitat parameter values obtained from the model with those evaluated in the field studies. The absolute value signs were added to make the gradient term independent of the direction of calculation. For all model results, the distance between V_1 and V_2 or v_1 and v_2 velocity measurements was 0.305 m, moving always along the x-axis (see Fig. 1). When these metrics are calculated along cross sections, V_1 and v_1 are always taken to be left (west) of V_2 and v_2. The metric values are also always plotted midway between the locations of V_1 and V_2 or v_1 and v_2. Hence, the metric values quantify the variations in the flow conditions occurring between the two velocity measurements in an averaged sense.

Spatial habitat metric values at fish locations

Hayes and Jowett (1994) and Fausch and White (1981) provided data that can be used to estimate the minimum and maximum possible values of $2V_{\mathrm{ave}} \times |(V_2 - V_1)/\Delta s|/v_{\mathrm{min}}^2$ that existed at the fish locations observed in their studies. Specifically, both studies provided a range of focal point velocity values (V_{min}), "velocity shears," and "velocity differences" that existed at fish locations. This information does not allow one to compute the exact parameter values found at each individual fish location. Only the minimum and maximum metric values that could have existed at the observed fish locations can be computed. The range of parameter values calculated here may therefore be substantially wider than those at which the fish were actually observed.

While Hayes and Jowett (1994) provided several data sets, only the optimal lateral velocity gradient data (using depth-averaged velocities) and the optimal vertical velocity gradient data (using point velocities) are analyzed here. These two data sets were collected at the locations of drift-feeding brown trout (*Salmo trutta*). The trout were 45–65 cm in length. Four data sets collected by Fausch and White (1981) were selected for analysis. These sets pertain to the allopatry resting and allopatry feeding locations of brook trout. Two of the data sets are for 15- to 20-cm trout locations; the other two are for 20- to 30-cm trout locations. The focal point velocities, velocity gradients, and Δs values used for each data set are shown in Table 1. The individual data sets will be referred to as data sets 1–6.

Results

Spatial flow patterns generated by the three modeling scenarios

In terms of depth, the flow fields of the first two simulations were very similar, regardless of whether or not bathymetry data on the boulders were incorporated into the model. The only significant differences in depth occurred immediately upstream of the four boulders at the head of the pool. There, depths increased on average by about 0.06 m, or by 10%. The second simulation (with all boulders) had a significantly different velocity field throughout the upper 25 m of the pool, particularly in the immediate vicinity of the boulders. Near the boulders, complex flow patterns existed that were absent in the first simulation. The differences in the velocity fields are illustrated by mapping the amount that the kinetic energy per unit mass ($v^2/2$) changes between the two simulations (Fig. 2). A change in kinetic energy of 0.100 $\mathrm{J \cdot kg^{-1}}$ corresponds to a change equal to 2.74 times the kinetic energy of water flowing at 0.27 $\mathrm{m \cdot s^{-1}}$ (the study site's average velocity), or 27% of the kinetic energy of water flowing at 0.86 $\mathrm{m \cdot s^{-1}}$ (the study site's maximum velocity).

The velocity fields generated within the 6 × 6 m box (shown in Fig. 1) for each modeling scenario are plotted in Fig. 3. Specifically, the y velocity component (v) within a 6 × 6 m square surrounding the location of the largest boulder is plotted for each scenario. Without boulders (Fig. 3*a*), the local flow pattern was fairly uniform except in one location where the presence of a small ridge on the channel bottom increased velocity values. The flow pattern around the single largest obstruction (Fig. 3*b*) was more complex. Steep velocity gradients adjacent to the boulder and a downstream wake were created. With all the boulders present (Fig. 3*c*), the surface plot became even more complex. It contained several velocity peaks and valleys, which provide several velocity magnitudes and velocity gradients that aquatic organisms can choose to inhabit.

Spatial habitat metric results

The three different model simulations produced distinctly different velocities and velocity gradients along the cross section taken just below the four boulders at the head of the pool (see Fig. 1). Figure 4*a* graphs the lateral y velocity profile for each model simulation. The velocity gradients corresponding to each of the velocity profiles are illustrated in

Table 1. Summary of the fish location data sets.

Data set	Study and conditions	Range of focal point velocities ($m \cdot s^{-1}$)	Range of velocity differences ($V_2 - V_1$) ($m \cdot s^{-1}$)	Range of velocity gradients (s^{-1})	Δs used between velocity measurements (m)
1	Hayes and Jowett (1994): lateral optimal	0.38–0.48	na	0.02–0.06	0.5 or 1.0
2	Hayes and Jowett (1994): vertical optimal	0.19–0.28	na	0.50–0.65	0.198–0.274
3	Fausch and White (1981): resting, 20–30 cm	0.095–0.169	0.348–0.384	na	0.60
4	Fausch and White (1981): feeding, 20–30 cm	0.156–0.210	0.217–0.393	na	0.60
5	Fausch and White (1981): resting, 15–20 cm	0.109–0.145	0.158–0.194	na	0.60
6	Fausch and White (1981): feeding, 15–20 cm	0.232–0.262	0.184–0.234	na	0.60

Note: na, not available.

Fig. 2. Change in kinetic energy per unit mass ($v^2/2$) induced by the presence of all of the exposed boulders. Color contours provide the magnitude of change in the kinetic energy per unit mass ($J \cdot kg^{-1}$). The area shown is the upper 25 m of the pool.

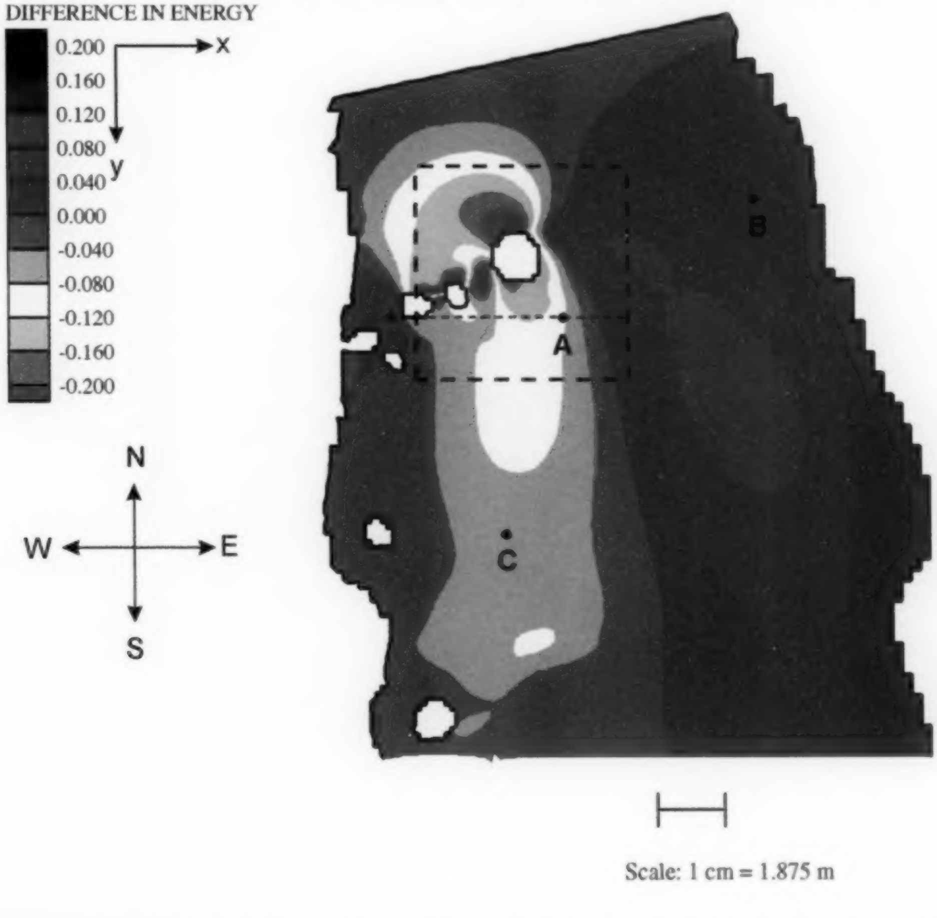
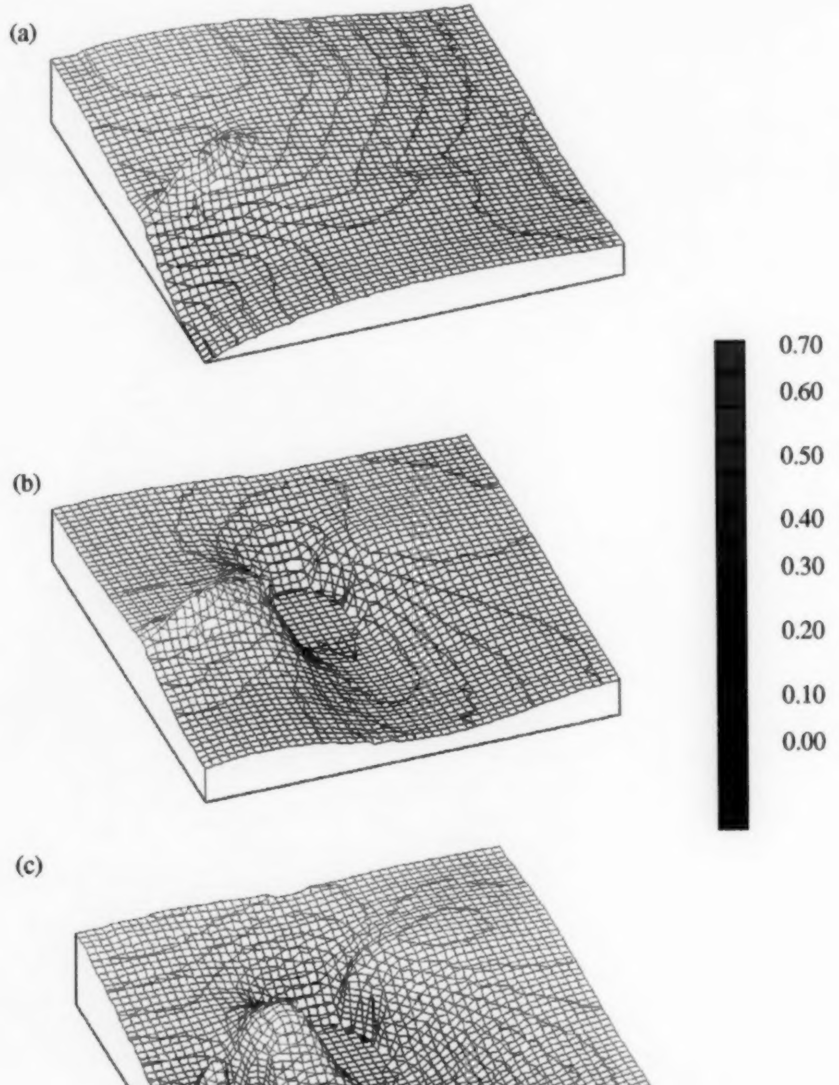


Fig. 4*b*. Without incorporating the boulders into the model, the velocity profile was relatively flat and had velocity gradients with absolute magnitudes of up to 0.122 s^{-1}. Model results obtained by incorporating only the single largest boulder into the channel geometry produced a sinuous velocity profile typical of a wake. Low velocity values existed immediately behind the boulder and high velocity values existed elsewhere. Associated with this velocity profile were velocity gradients having absolute magnitudes of up to 0.582 s^{-1}. With all of the boulders included, the velocity profile became even more sinuous and local velocity gradients had absolute magnitudes of up to 0.692 s^{-1}, which is 5.67 times those predicted in their absence.

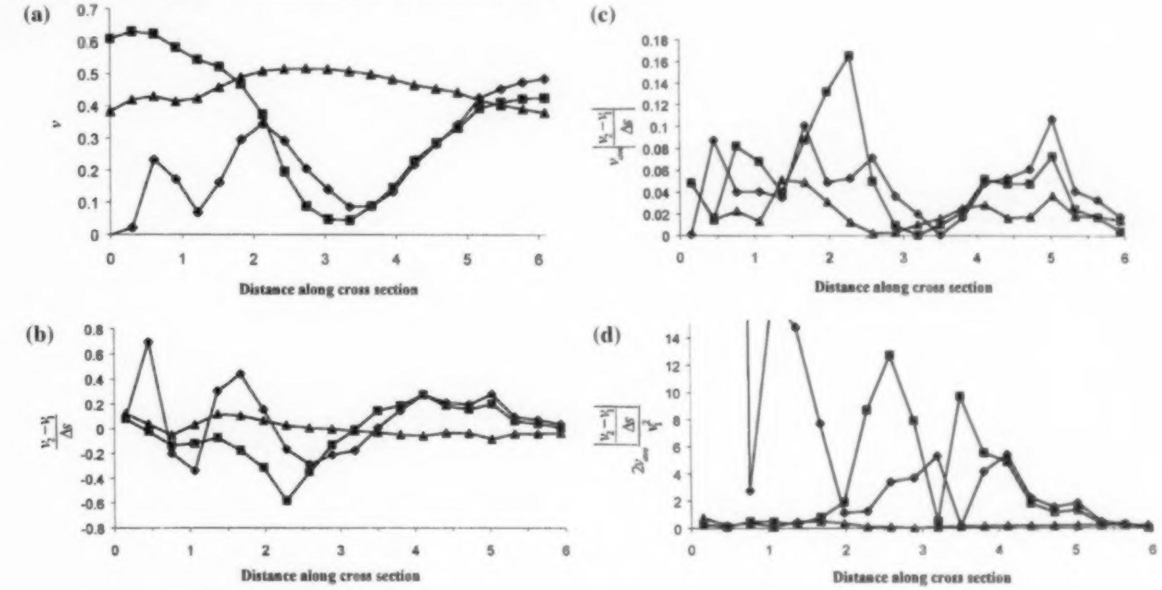
The range of velocity and velocity gradient magnitudes associated with the three simulations produced a wide range of spatial change in kinetic energy ($v_{ave} \times |(v_2 - v_1)/\Delta s|$) val-

Fig. 3. Surface plots of the y velocity component found within the 6 × 6 m square shown in Fig. 2. The surface plot obtained without boulders is shown in Fig. 3*a*; the surface plots obtained for the single largest boulder and all of the boulders are shown in Figs. 3*b* and 3*c*, respectively. Color contours represent velocity in $\mathrm{m \cdot s^{-1}}$. The flat locations having zero velocity in Figs. 3*a* and 3*b* indicate the locations of boulders.



ues. Figure 4*c* plots $v_{\mathrm{ave}} \times |(v_2 - v_1)/\Delta s|$ along the cross section for each model scenario. Without including boulder geometry into the model, the spatial change in kinetic energy was generally lower than that predicted when either the single boulder or all of the boulders were incorporated into the model. The maximum spatial change in kinetic energy per unit length in the absence of boulders was 0.051 $\mathrm{J \cdot kg^{-1} \cdot m^{-1}}$ and represented only 0.31 and 0.47 times the maximum spatial change in kinetic energy computed for the single- and multiple-boulder scenarios, respectively. The maximum change in kinetic energy per unit length in the profiles for the single obstruction was 0.165 $\mathrm{J \cdot kg^{-1} \cdot m^{-1}}$ and occurred at a location in the boulder's wake, which had a velocity of approximately 0.29 $\mathrm{m \cdot s^{-1}}$. An organism at that location has ac-

Fig. 4. Graphs of (*a*) velocity, (*b*) velocity gradient, (*c*) $v_{ave} \times |(v_2 - v_1)/\Delta s|$, and (*d*) $2v_{ave} \times |(v_2 - v_1)/\Delta s|/v^2_{min}$ along the cross section shown in Fig. 1 for each model simulation. Distance is measured from the west end of the line passing through point A (see Fig. 1). The diamonds indicate values obtained from the simulation containing all of the boulders. The squares and triangles represent values obtained from the simulations containing the single boulder and no boulders, respectively. Velocity gradient, $v_{ave} \times |(v_2 - v_1)/\Delta s|$, and $2v_{ave} \times |(v_2 - v_1)/\Delta s|/v^2_{min}$ are plotted midway between v_1 and v_2.



cess to flow within 0.30 m that has 2.18 times more kinetic energy passing through its location, or 1.48 times its focal point velocity. With all of the obstructions present, the maximum change in kinetic energy was 0.11 $J \cdot kg^{-1} \cdot m^{-1}$ and occurred at a location having a velocity of 0.39 $m \cdot s^{-1}$. A spot 0.30 m away would have 1.43 times more kinetic energy than the original location.

Because identical values of $v_{ave} \times |(v_2 - v_1)/\Delta s|$ can be obtained with different combinations of velocity and velocity gradient (e.g., small v and large $|(v_2 - v_1)/\Delta s|$ or large v and small $|(v_2 - v_1)/\Delta s|$), it was difficult to visually determine from Fig. 4*c* which velocity profile exhibits the most spatial variability. A plot of $2v_{ave} \times |(v_2 - v_1)/\Delta s|/v^2_{min}$, however, clearly identified the locations along the three velocity profiles where significant spatial variations are present (Fig. 4*d*). The values of this metric along the profile generated without obstructions were small (ranging from 0.01 to 0.67 m^{-1}) everywhere along the profile. Such results are consistent with what one expects of relatively uniform flow conditions. The values of the parameter ranged from 0.04 to 9.69 m^{-1} along the profile for the single-boulder simulation. The larger of these values occurred in the wake region behind the boulder. This is expected. These locations correspond to areas having relatively low velocities and high velocity gradients. The parameter values obtained for the profile generated with all the boulders incorporated into the model ranged from 0.15 to 393.59 m^{-1}. As with the profile obtained with the single boulder, the larger values were typically found in the wake regions created by the boulders. The largest values (393.59 and 97.00 m^{-1}) were located immediately next to a boulder. These large values can be expected extremely close to banks or boulders and are triggered by the very small V_{min} values at these locations. A comparison of the three profiles shown in Fig. 4*d* quickly reveals that the velocity profiles obtained with the single boulder and with all of the boulders have significantly more spatial variability than the profile obtained without boulders.

The spatial parameters $v_{ave} \times |(v_2 - v_1)/\Delta s|$ produced values that differed by a factor of 4 between points A and C. Because the velocity is the same at these three points, the values of $2v_{ave} \times |(v_2 - v_1)/\Delta s|/v^2_{min}$ will differ by the same factor as the first parameter values. The velocity, velocity gradient, and habitat metric values at points A, B, and C are summarized in Table 2. The highest parameter values occurred at point A, which is surrounded by the steep velocity gradients and complex flow patterns generated by boulders (Fig. 1). The lowest parameter values were found at location C, which is in the middle of a velocity shelter where flow conditions are not changing quickly. Point B, near the east bank where velocity values are changing fairly quickly, created intermediate parameter values. Such results are expected. Since the parameter values range by a factor of 4, the metrics appear to provide a useful means of distinguishing between various flow conditions within the study site. The velocity at points A, B, and C falls within the range of focal velocities observed for the fish in field data set 4. Therefore, points A, B, and C represent potential feeding locations of 20- to 30-cm brook trout. As shown later, point C has the metric value closest to those by which the trout in data set 4 were surrounded.

The velocity magnitude, V, is often of primary importance in stream management issues. Consequently, the parameters $V_{ave} \times |(V_2 - V_1)/\Delta s|$ and $2V_{ave} \times |(V_2 - V_1)/\Delta s|/v^2_{min}$ were plotted along the cross section below the boulders for each

Table 2. Habitat metrics at points A, B, and C when all boulders are modeled.

Point	v (m·s^{-1})	$\lvert(v_2 - v_1)/\Delta s\rvert$ (s^{-1})	$v_{ave} \times \lvert(v_2 - v_1)/\Delta s\rvert$ (J·kg^{-1}·m^{-1})	$2v_{ave} \times \lvert(v_2 - v_1)/\Delta s\rvert/v_1^2$	Representative flow condition
A	0.200	0.249	0.050	2.487	Boulder's wake
B	0.199	0.106	0.021	1.065	Unprotected bank
C	0.200	0.052	0.010	0.520	Velocity shelter

Fig. 5. Graphs of (*a*) $V_{ave} \times |(V_2 - V_1)/\Delta s|$ and (*b*) $2V_{ave} \times |(V_2 - V_1)/\Delta s|/v_{min}^2$ along the cross section shown in Fig. 1 for each model simulation. Distance is measured from the west end of the line passing through point A (see Fig. 1). The diamonds indicate values obtained from the simulation containing all of the boulders. The squares and triangles represent values obtained from the simulations containing the single boulder and no boulders, respectively. The metric values $V_{ave} \times |(V_2 - V_1)/\Delta s|$ and $2V_{ave} \times |(V_2 - V_1)/\Delta s|/v_{min}^2$ are plotted midway between V_1 and V_2.

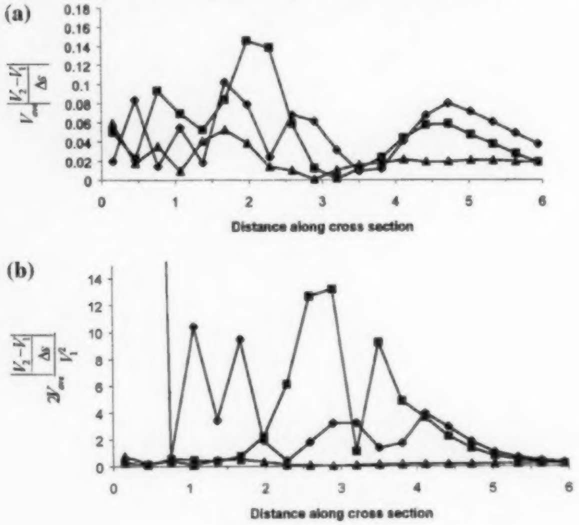
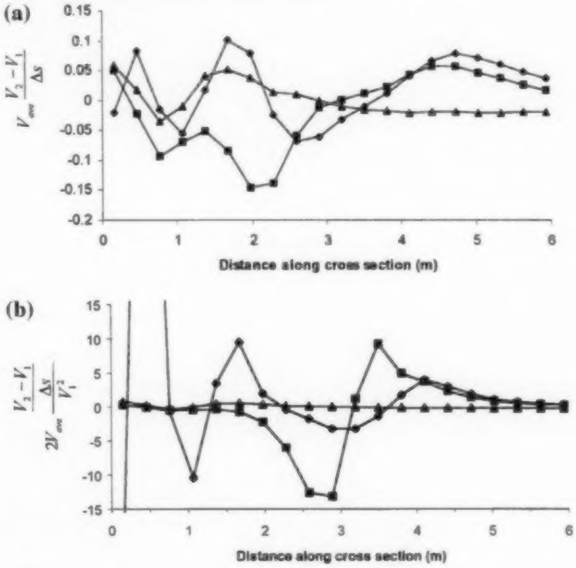


Fig. 6. Graphs of (*a*) $V_{ave} \times (V_2 - V_1)/\Delta s$ and (*b*) $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ along the cross section shown in Fig. 1 for each model simulation. Distance is measured from the west end of the line passing through point A (see Fig. 1). The diamonds indicate values obtained from the simulation containing all of the boulders. The squares and triangles represent values obtained from the simulations containing the single boulder and no boulders, respectively. The metric values $V_{ave} \times (V_2 - V_1)/\Delta s$ and $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ are plotted midway between V_1 and V_2.



simulation (Fig. 5). The profiles generated using $V_{ave} \times |(V_2 - V_1)/\Delta s|$ and $2V_{ave} \times |(V_2 - V_1)/\Delta s|/v_{min}^2$ are similar to those obtained with $v_{ave} \times |(v_2 - v_1)/\Delta s|$ and $2v_{ave} \times |(v_2 - v_1)/\Delta s|/v_{min}^2$ (Figs. 4 and 5), except for some differences that exist in certain locations. These differences occur in locations where v is significantly less than V. A comparison of the y velocity components with velocity magnitudes along the cross sections revealed that on average, the y velocity component constitutes over 96% of the velocity magnitude for the simulations with the single boulder and no boulders. With all the boulders present, the y velocity component represented on average 84% of the velocity magnitude. These high percentages suggest that, in this case, v can be used to approximate V and will produce similar habitat parameters along most of the cross section.

The absolute values in the spatial metrics plotted in Fig. 5 render the energy gradients independent of direction. This independence is useful when analyzing the magnitudes of the energy gradients found within a study reach. One may wish to remove the absolute value signs, as this provides a more descriptive representation of how the flow's energy is changing throughout a river. The metrics $V_{ave} \times (V_2 - V_1)/\Delta s$ and $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ are plotted for each model scenario in Figs. 6*a* and 6*b*, respectively. The plots of $V_{ave} \times (V_2 - V_1)/\Delta s$ for all three simulations are sinuous and do not clearly reveal which simulations had significant variations in their flow patterns. However, the plots of $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ clearly distinguish the spatially varying flow patterns found in the single- and multiple-boulder simulations from the uniform flows found in the no-boulders simulation. With no boulders present, $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ plots almost as a straight line with values approaching zero. In contrast, the profiles for the single- and multiple-boulder simulations are quite sinuous. As the metric values were calculated from left to right (V_1 always being left (west) of V_2), a positive value of $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ indicates that the flow's energy is increasing from west to east at that point. A negative value of $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ indicates that the flow's energy is decreasing from west to east at that point in the river. The magnitude of the parameter represents the relative rate at which the energy is changing.

Table 3. Metric values generated for the numerical simulations and fish location data.

Data set	Study conditions	V_1	V_2	Δs	V_{ave}	$\lvert V_2 - V_1/\Delta s\rvert$	$2V_{ave} \times \lvert(V_2 - V_1)/\Delta s\rvert/V_1^2$	Extrema
1	Hayes and Jowett (1994): lateral	0.380	0.440	1.000	0.410	0.060	0.341	Maximum
		0.480	0.500	1.000	0.490	0.020	0.085	Minimum
		0.380	0.410	0.500	0.395	0.060	0.328	Maximum
		0.480	0.490	0.500	0.485	0.020	0.084	Minimum
2	Hayes and Jowett (1994): vertical	0.190	0.368	0.274	0.279	0.650	10.049	Maximum
		0.280	0.417	0.274	0.349	0.500	4.445	Minimum
		0.190	0.319	0.198	0.254	0.650	9.159	Maximum
		0.280	0.379	0.198	0.330	0.500	4.203	Minimum
3	Fausch and White (1981): resting, 20–30 cm	0.095	0.479	0.600	0.287	0.640	40.705	Maximum
		0.169	0.517	0.600	0.343	0.580	13.931	Minimum
		0.095	0.290	0.305	0.193	0.640	27.316	Maximum
		0.169	0.346	0.305	0.257	0.580	10.456	Minimum
4	Fausch and White (1981): feeding, 20–30 cm	0.156	0.549	0.600	0.353	0.655	18.975	Maximum
		0.210	0.427	0.600	0.319	0.362	5.224	Minimum
		0.156	0.356	0.305	0.256	0.655	13.774	Maximum
		0.210	0.320	0.305	0.265	0.362	4.349	Minimum
5	Fausch and White (1981): resting, 15–20 cm	0.109	0.303	0.600	0.206	0.323	11.212	Maximum
		0.145	0.303	0.600	0.224	0.263	5.611	Minimum
		0.109	0.208	0.305	0.158	0.323	8.617	Maximum
		0.145	0.225	0.305	0.185	0.263	4.638	Minimum
6	Fausch and White (1981): feeding, 15–20 cm	0.232	0.466	0.600	0.349	0.390	5.058	Maximum
		0.262	0.446	0.600	0.354	0.307	3.163	Minimum
		0.232	0.351	0.305	0.291	0.390	4.224	Maximum
		0.262	0.356	0.305	0.309	0.307	2.759	Minimum
7	Present study: no boulders	0.400	0.444	0.305	0.422	0.140	0.738	Maximum
		0.524	0.524	0.305	0.524	0.001	0.002	Minimum
8	Present study: single boulder	0.050	0.097	0.305	0.074	0.156	13.155	Maximum
		0.643	0.632	0.305	0.637	0.036	0.112	Minimum
9	Present study: all boulders	0.041	0.229	0.305	0.135	0.616	97.350	Maximum
		0.495	0.517	0.305	0.506	0.073	0.304	Minimum

Had the values been calculated from right (east) to left (west), all the metric values would have the same magnitude but would be opposite in sign.

Spatial habitat metric values at observed fish locations

The minimum possible value of $2V_{ave} \times \lvert(V_2 - V_1)/\Delta s\rvert/v_{min}^2$ for each fish habitat data set would occur if the mildest velocity gradient surrounded the fish location having the fastest focal point velocity. The maximum possible value would occur if the steepest velocity gradient existed at the location having the slowest focal point velocity. The minimum and maximum possible parameter values at the Δs values used to collect data sets 1–6 are given in Table 3. Values at Δs = 0.305 m were estimated for comparison with the results obtained along the cross section in each model simulation.

The largest parameter values, ranging from 10.456 to 27.316 m^{-1}, were obtained from data set 3. These values are typically much larger than those found in the three model simulations. Only one point in the all-boulders simulation had a parameter value higher than the values found in data set 3. That point had a value of 97.350 m^{-1} and occurred in the all-boulders simulation. Excluding the 97.350 m^{-1} value and a value of 22.850·m^{-1} (found in very slow water near a boulder), the parameter values range from 0.304 to 10.416 m^{-1} along the cross section shown in Fig. 1. For the simulations containing the single-boulder and no-boulders cases, the corresponding ranges are 0.116–13.155 and 0.002–0.738 m^{-1}. Only two values (12.624 and 13.155 m^{-1}) fall within range of 10.456–27.316 m^{-1}. This suggests that the energy gradients along the cross section are routinely smaller than those used by the trout observed in data set 3. The lowest metric values were obtained from data set 1. The parameter values range from 0.084 to 0.328 m^{-1} and coincide most closely with the values found in the model simulation containing no boulders. The ranges of parameter values found in data sets 2, 4, 5, and 6 are similar to each other. Combined, the data sets have parameter values ranging from 2.759 to 13.774 m^{-1}. These values overlap with the ones predicted by the model for the single- and multiple-boulder simulations (0.116–13.155 m^{-1}). The likeness of these two ranges suggests that the flow patterns in the single- and multiple-boulder simulations may provide suitable habitat for the fish studied in data sets 2, 4, 5, and 6.

Discussion

Two-dimensional hydraulic models provide stream managers with spatially explicit information about flow patterns within a study reach, including flow direction, velocity gradients, velocity shelters, and transverse flows. If such two-dimensional features provide essential, or even preferred, habitat conditions, then the accurate quantification of the number and type of these features may lead to improved instream flow analyses and stream restoration techniques.

Despite recent progress in using two-dimensional models in habitat suitability studies, substantial research remains to be performed to determine the accuracy with which one can predict the complex flow patterns that natural streams exhibit. Differences between implementing one-dimensional and two-dimensional models make it difficult to compare the labor costs and model accuracy associated with one-dimensional and two-dimensional models. Both the labor required and the accuracy of particular model depend on the individual site being studied. Some researchers (e.g., Ghanem et al. 1996) suggest, but have not conclusively shown, that the collection of field data for two-dimensional hydraulic models may be less intensive than that needed to run and calibrate PHABSIM. One reason for this belief is that the more intensive bathymetry data required by two-dimensional models can be collected faster than the velocity data needed to run PHABSIM simulations.

The type of spatial variability that is important to quantify may depend on both the species and the life stage of the organism being studied. The spatial habitat metrics $2V_{\mathrm{ave}} \times (V_2 - V_1)/\Delta s/v^2_{\mathrm{min}}$ and $2v_{\mathrm{ave}} \times (v_2 - v_1)/\Delta s/v^2_{\mathrm{min}}$ were found to be capable of describing and differentiating between various flow conditions within the study site. In this case, the direction of the line between velocities 1 and 2 was in the x direction. Therefore, the parameter describes lateral velocity and energy gradients. Such a parameter has the potential to identify the habitat of drift-feeding fish, which often use the lateral velocity/energy gradients generated by boulders as feeding stations. However, longitudinal velocity/energy gradients may be a more useful means of quantifying other habitats. Foraging fish may find longitudinal velocity gradients, such as those located between the tail of a pool and the head of a riffle, to be an important habitat feature. Likewise, the evaluation of potential salmon spawning grounds may benefit from an analysis of local longitudinal velocity gradients, as it may be indicative of the flow's capacity to prevent siltation from destroying the redd. In other cases, fish may be using vertical velocity/energy gradients to feed. Fortunately, the parameter $2V_{\mathrm{ave}} \times (V_2 - V_1)/\Delta s/v^2_{\mathrm{min}}$ is general in nature and the line between the two velocity measurements can be in the x, y, or z (or any other desirable) direction. If the x or y velocity components are important characteristics, then the substitution of these velocities into the parameter may also lead to an appropriate means of quantifying stream habitat.

All of the variations of the parameter described above reflect spatial changes in energy and the percent increase/decrease in energy expenditure levels that an organism will encounter by moving in a specific direction of the flow. Consequently, the amount of energy that a fish or other organism must expend to move to a nearby location, as well as the amount of kinetic energy that the nearby flow has available for bringing in food, can be calculated. These spatial changes in energy are also indicative of the degree to which flow patterns are changing around a location. The faster the flow is changing spatially, the higher these values will be. Such information, combined with focal point velocity and depth information, may lead to better suitability criteria. For example, adding velocity/energy gradient values into habitat suitability criteria would allow one to distinguish between a fairly uniform flow and a highly spatial flow pattern having similar focal depth and velocity values. Each of these different habitats can then be weighted appropriately.

The values of $2V_{\mathrm{ave}} \times |(V_2 - V_1)/\Delta s|/v^2_{\mathrm{min}}$ for the single- and multiple-boulder simulations are similar to the magnitudes found at fish locations observed by Hayes and Jowett (1994) and Fausch and White (1981) (data sets 2, 4, 5, and 6). These comparisons, however, are based on the maximum and minimum possible values and not on the actual parameters observed at each individual fish location. The direction and distance between the velocity measurements also varied between and within the studies, making a complete comparison difficult. Input from stream biologists is needed to determine if particular velocity/energy gradients (vertical, lateral, or longitudinal) best describe a specific habitat. Field data need to be collected to determine what parameter values can be expected to be found surrounding a particular species and age/size group. The distances between the velocity measurements necessary to calculate these parameter values need to be established. A distance that is too short or too long will not describe the velocity/energy gradient of importance and under- or over-estimate local gradients. One possible approach is to space the velocity measurements a distance l apart, where l is a distance related to the length of the fish or the diameter of the boulder generating the flow pattern. Spacing related to the fish length or boulder diameter allows one to multiply l by $2V_{\mathrm{ave}} \times (V_2 - V_1)/\Delta s/v^2_{\mathrm{min}}$ and obtain a dimensionless parameter. Dimensionless parameters have the potential to unify stream habitat suitability criteria when comparing data corresponding to different species, ages, and size-classes. Moreover, a distance related to the fish length seems appropriate, as it can be related to the distance that a fish may be expected to travel to obtain food and the area that it may defend.

The additional data needed to incorporate the proposed spatial habitat metric into a study depend on which velocity gradients are important and how the velocity gradients are measured. If an approach similar to that used by Hayes and Jowett (1994) and Fausch and White (1981) is followed, velocity should be measured at the focal point and at a point in the direction of the gradient of interest. If longitudinal or lateral velocity gradients are important, then depth-averaged velocity measurements provide a means of directly relating the resulting habitat data to two-dimensional model output. For vertical velocity gradients, point velocities must be measured and a vertical velocity profile assumed in the two-dimensional model output, if two-dimensional model output is to be used to help quantify useable habitat. Depending on the habits of a particular organism, this information may be needed for the different locations that the fish or organism routinely uses (e.g., resting and feeding locations).

In conclusion, flow patterns such as velocity gradients, recirculation zones, and transverse flows typically generated by the presence of mesoscale topography play an important role in stream ecology. Point measurements (focal depth, velocity, substrate, and cover) are inadequate to characterize and differentiate between these types of spatially varying flow features. Consequently, metrics that are capable of capturing the spatial flow characteristics need to be developed.

Spatial metrics, combined with focal point depth, velocity, substrate, and cover values, will more fully describe local flow conditions and therefore might lead to better habitat suitability criteria. This emphasizes the need for including boulders and other obstructions when simulating stream flows with the use of two-dimensional numerical models. Spatially explicit output provided by such models can be employed to better assess the quantity and quality of habitat within a stream.

The parameters $V_{ave} \times (V_2 - V_1)/\Delta s$ and $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ were tested as potential spatial habitat metrics that can be used with field data and two-dimensional models. Both parameters quantify velocity gradients and changes in kinetic energy that are associated with spatially varying flows. The parameter $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$, however, better quantified and distinguished between locations having high spatial variation and those having more uniform flow conditions. Locations near the bank and exposed boulders, which had high spatial velocity variability, a feature known to be characteristic of natural streams having good habitat, produced the highest absolute values of $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$. Locations within stream segments without boulders typically exhibited low spatial velocity variability and resulted in significantly lower absolute values of $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$.

The spatial habitat metric $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$ is indicative of the average rate of change in kinetic energy between two points scaled by the kinetic energy at the point having the smaller velocity. The parameter can be adapted to quantify vertical, lateral, or longitudinal velocity gradients, depending on which gradient best describes a specific habitat. As the parameter is directly related to a flow's kinetic energy, it can facilitate the computation of fish energy expenditure rates and locate potential feeding and resting stations.

The parameter $2V_{ave} \times (V_2 - V_1)/\Delta s/v_{min}^2$, while capable of quantifying spatial changes in kinetic energy, is only one choice of a spatial habitat metric. Other spatial habitat metrics characterizing different spatially varying flow properties may be devised and chosen to develop habitat suitability criteria. Identifying the most appropriate parameters for a given circumstance needs further research. However, the ability to accurately quantify spatially varying flow features important to aquatic flora and fauna can facilitate instream flow decisions and provide much needed information for stream restoration projects. The results presented here can be used as a guide to future research efforts toward attaining that objective.

Acknowledgements

We gratefully acknowledge the support provided by Pacific Gas and Electric, the National Science Foundation, and the United States Environmental Protection Agency. Several insightful comments, provided by Lane Watson and two anonymous reviewers, helped improve the overall presentation and expand the scope of this work.

References

Biggs, J.F., Duncan, M.J., Francoeur, S.N., and Meyer, W.D. 1997. Physical characterisation of microform bed cluster refugia in 12 headwater streams. N.Z. J. Mar. Freshwater Res. **13**: 413–422.

Bovee, K.D. 1978. The incremental method of habitat potential for coolwater species with management implications. Am. Fish Soc. Spec. Publ. **11**: 340–346.

Crowder, D.W., and Diplas, P. 2000. Using two-dimensional hydrodynamic models at scales of ecological importance. J. Hydrol. **230**(3–4): 172–191..

Fausch, K.D., and White, R.J. 1981. Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for position in a Michigan stream. Can. J. Fish. Aquat. Sci. **38**: 1220–1227.

Ghanem, A., Steffler, P., Hicks, F., and Katopodis, C. 1996. Two-dimensional hydraulic simulation of physical habitat conditions in flowing streams. Regul. Rivers Res. Manage. **12**: 185–200.

Hayes, J.W., and Jowett, I.G. 1994. Microhabitat models of large drift-feeding brown trout in three New Zealand rivers. N. Am. J. Fish. Manage. **14**: 710–725.

King, I.P. 1990. Program documentation RMA-2V, two dimensional finite element model for flow in estuaries and streams, version 4.3. Resource Management Associates, Lafayette, Calif.

Lancaster, J., and Hildrew, A.G. 1993. Flow refugia and the microdistriubtion of lotic macroinvertebrates. J. North Am. Benthol. Soc. **12**: 385–393.

Leclerc, M., Boudreault, A., Bechara, J.A., and Corfa, G. 1995. Two-dimensional hydrodynamic modeling: a neglected tool in the instream flow incremental methodology. Trans. Am. Fish. Soc. **124**: 645–662.

Milhous, R.T., Updike, M.A., and Schneider, D.M. 1989. Physical habitat simulation system (PHABSIM) reference manual, version II. Instream Flow Inf. Pap. No. 26. U.S. Fish Wildl. Serv. Biol. Rep. 89(16).

Munson, B.R., Young, D.F., and Okiishi, T.H. 1990. Fundamentals of fluid mechanics. 2nd ed. John Wiley & Sons, Inc., New York.

Rader, R.B., and Poff, N.L. 1999. Development of an individual-based trout instream flow model. Stream Notes. Stream Systems Technology Center, USDA Forest Service, Rocky Mountain Research Center, Fort Collins, Colo.

Rempel, L.L., Richardson, J.S., and Healey, M.C. 1999. Flow refugia for benthic macroinvertebrates during flooding of a large river. J. North Am. Benthol. Soc. **18**: 34–48.

Tarbet, K., and Hardy, T.B. 1996. Evaluation of one-dimensional and two-dimensional hydraulic modeling in a natural river and implications in instream flow assessment methods. *In* Ecoydraulics 2000. Proceedings of the second International Symposium on Habitat Hydraulics. Vol. B. *Edited by* M. Leclerc, H. Capra, S. Valentin, A. Boudreault, and Y. Côté. INRS-Eau and FQSA, IAHR/AIRH, Québec, Qué. pp. B395–B406.

Effects of riffle length on the short-term movement of fishes among stream pools

D.G. Lonzarich, M.R. Lonzarich, and M.L. Warren, Jr.

Abstract: Recent research has suggested that the within-habitat dynamics of fish populations and assemblages can be affected by the spatial distribution of habitats within streams. In this study, we determined the extent to which pool isolation (length of riffles connecting adjacent pools) influenced fish movement in two Arkansas streams. We marked individuals from 12 pools assigned to two treatment categories: pools separated by long riffles (>50 m) and those separated by short riffles (<10 m). Repeatedly snorkeling pools for 3 days in 1995 and 1997, we discovered substantial emigration (>20%) and significant effects of riffle length. Total emigration from short-riffle pools was three times higher (29%) than movement from long-riffle pools (10%). Further, marked fish in short-riffle pools moved upstream and downstream with equal frequency, whereas fish in long-riffle pools moved twice as often downstream. Collectively, these results indicate significant effects of habitat spacing on short-term movement patterns by fish. In streams, where fish are distributed within a mosaic of habitats of varying quality, such movements may allow individuals to assess spatial variability in resource conditions (e.g., food, predators). Because land-use activities can alter habitat spacing, these findings have important implications for fish conservation in degraded streams.

Résumé : Des recherches récentes semblent montrer que la dynamique des populations et des assemblages de poissons au sein des habitats peut être influencée par la répartition spatiale des habitats dans les cours d'eau. Dans la présente étude, nous avons déterminé dans quelle mesure l'isolement des fosses (longueur des radiers reliant des fosses contiguës) influait sur le déplacement des poissons dans deux cours d'eau de l'Arkansas. Nous avons marqué les individus prélevés dans 12 fosses réparties en deux catégories expérimentales : fosses séparées par des radiers longs (>50 m) et fosses séparées par des radiers courts (<10 m). Des opérations répétées d'observation en plongée avec masque et tube pendant 3 jours, en 1995 et 1997, nous ont permis de découvrir une émigration importante (>20%) et des effets significatifs liés à la longueur des radiers. L'émigration totale des fosses à radiers courts était trois fois plus grande (29%) que les déplacements des poissons des fosses à radiers longs (10%). De plus, les poissons marqués dans les fosses à radiers courts se déplaçaient vers l'amont et vers l'aval avec une fréquence égale, tandis que les poissons des fosses à radiers longs se déplaçaient deux fois plus souvent vers l'aval. Collectivement, ces résultats indiquent que l'espacement des habitats a des effets significatifs sur les patrons de déplacement à court terme des poissons. Dans les cours d'eau, où les poissons sont répartis dans une mosaïque d'habitats de qualité variable, de tels déplacements peuvent permettre aux individus d'évaluer la variabilité spatiale de l'état des ressources (p. ex. la nourriture, les prédateurs). Étant donné que les activités d'aménagement des terres peuvent modifier l'espacement des habitats, ces résultats ont des incidences importantes pour la conservation des poissons dans les cours d'eau dégradés.

[Traduit par la Rédaction]

Introduction

Biologists have long been interested in the movement of fish in streams. Studies on movement contribute towards understanding the habitat needs of different species (reviewed by Gowan et al. 1994) and in delineating the spatial boundaries of populations (e.g., Hill and Grossman 1987; Freeman 1995). Additionally, knowledge of fish movement patterns can be useful in predicting the effects of physical barriers, which in streams exist in many forms. For example, persistent barriers such as waterfalls and debris dams can block migratory movements of fish, limiting access to stream reaches (e.g., Kruse et al. 1997) or restricting gene flow between populations (e.g., Congdon 1995; Fausch and Young 1995). In intermittent streams, dry riffle habitats create temporary barriers to interhabitat movement that can affect species richness and incidence patterns within isolated pool habitats (e.g., Taylor 1997).

To a lesser, although potentially important, extent, pool isolation also can arise in perennial streams where habitats are organized into discrete patches. Under such conditions, differences in water depth and current between pools and riffles may affect the degree to which pool-dwelling fishes traverse riffles to access neighboring pools. Gerking (1953) was probably the first to discuss this possibility when he proposed that long riffles separating adjacent pool habitats could act as behavioral barriers to movement and that fishes within individual pools could be viewed as discrete populations. Matthews et al. (1994) also speculated that riffles act as size-selective barriers to movement and affect pool as-

Received October 25, 1999. Accepted March 31, 2000. J15421

D.G. Lonzarich[1] and M.R. Lonzarich. Department of Biology, University of Wisconsin - Eau Claire, Eau Claire, WI 54702, U.S.A.
M.L. Warren, Jr. USDA Forest Service, Forest Hydrology Laboratory, 1000 Front St., Oxford, MS 38655, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: lonzard@uwec.edu

semblage dynamics. Recent experiments have added weight to these earlier observations by showing that long riffles slow fish recolonization to defaunated pools (Lonzarich et al. 1998) and reduce fish movement between pools in experimental streams (Schaefer 1999).

Unlike waterfalls, riffles are neither permanent nor absolute barriers to fish movement. However, riffles are a much more common type of barrier in streams, and under certain conditions, their affects on interhabitat movement may lead to important ecological consequences. Because differences between stream habitats tend to be greatest during base flows, it may be at these times (e.g., summer) that riffles, especially long riffles, become effective barriers. While not preventing movement, long riffles may significantly reduce daily excursions between habitat patches and limit the ability of fish to track variability in food resources and predator densities.

The potential impacts of habitat patchiness may be most severe in degraded streams where land-use activities modify stream channels or reduce the availability of habitat-forming woody debris (e.g., Karr and Schlosser 1978; Keller and Swanson 1979; Schlosser 1991). Such changes can reduce the frequency of pool habitats (Ralph et al. 1994), which in turn increases interpool distances (e.g., Beechie and Sibley 1997). Although many ecological impacts have been linked to land-use changes, much of the relevant research has focused on habitat-units (e.g., pools) or stream-scale responses. While recent research has begun to address the effects of land-use activities on habitat connectivity, fragmentation, and heterogeneity (e.g., Schlosser 1991, 1995; Ward 1998), the emphasis is on large spatial scales (i.e., riverine landscapes) and moderately long time scales (e.g., generations).

We strongly believe that efforts to better understand the ecology of stream fishes and, more specifically, the consequences of land-use activities will benefit from research aimed at addressing the relationship between fish movement and habitat patchiness. We undertook this study to characterize short-term, small-scale movements by a suite of pool-dwelling fishes in two Arkansas streams and to identify the extent to which these movements were affected by pool isolation (defined here by the length of riffles connecting adjacent pools). Our specific objectives were to (*i*) quantify fish movement over a 3-day period and (*ii*) describe whether pool isolation affected the degree or direction of overall and species-specific movement.

Materials and methods

We carried out this study in August of 1995 and 1997 in two tributary streams of the Little Missouri River in the Ouachita National Forest, Arkansas (Fig. 1). Long and Blaylock creeks are relatively short (<10 km), moderate-gradient systems that flow through predominantly forested and mountainous terrain. General characteristics of the streams included bedrock and gravel substrates and dense riparian vegetation. The proportion and average size of pool and riffle habitats in the two streams were similar (Clingenpeel 1994) as were the composition, rank dominance, and densities of fish species (Lonzarich et al. 1998). Physical and biological conditions during the two years of the study also were very similar. Flow conditions, as reflected by average riffle depth, did not differ between the two years (two-sample *t* test, $P = 0.87$). Further, the relative abundances of the five target species during the 1995 and 1997 surveys were very highly correlated (Spearman rank test, $r = 0.90$).

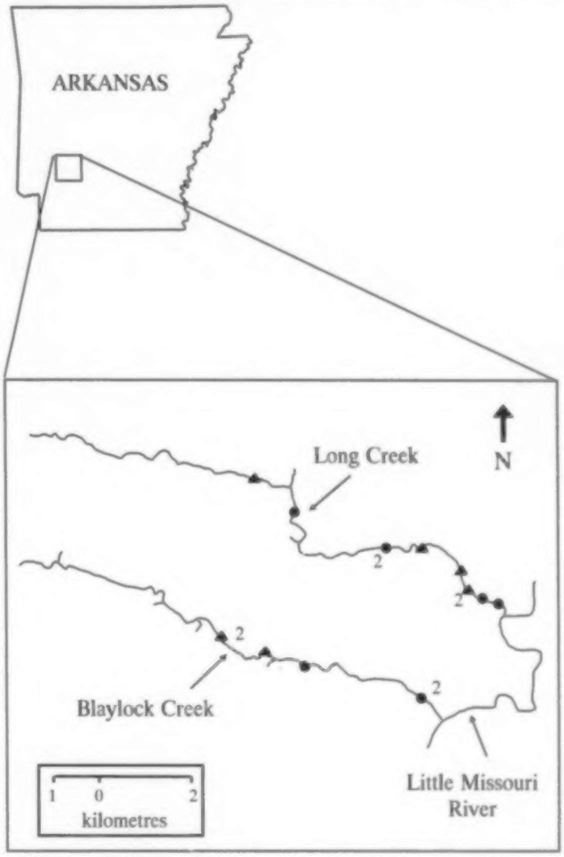


Fig. 1. Location of short-riffle (●) and long-riffle (▲) pools in Long and Blaylock creeks (34°22′30″N, 93°52′30″W). Sites labeled with the number "2" were included during both survey years.

Experimental design

We assigned 12 pools from the two streams equally into two different treatment categories based on riffle length. Short-riffle pools were separated from adjacent upstream and downstream pools by riffles <10 m long (mean = 8.1 m, 1 SE = ±1.5), and long-riffle pools were separated from adjacent pools by riffles ≥25 m long (mean = 50 m, 1 SE = ±12.2) (Table 1). With the exception of these differences, the average physical dimensions of pools in the two treatment groups were similar (two-sample *t* test, $P > 0.10$) (Table 1). Of the six pools in each treatment, two were surveyed in both 1995 and 1997. Although it was unlikely that the same individuals were collected in both years, we nonetheless combined the 1995 and 1997 data from these pools; thus, each treatment consisted of six replicated pools.

We marked a subset of the fish species that commonly occur in the two streams. We did not mark juvenile fish (<50 mm total length) or cryptic bottom-dwelling species, which are difficult to locate using visual survey techniques. We included central stoneroller (*Campostoma anomalum*), striped shiner (*Luxilus chrysocephalus*), northern studfish (*Fundulus catenatus*), longear sunfish (*Lepomis megalotis*), and smallmouth bass (*Micropterus dolomieu*).

Table 1. Physical characteristics of short-riffle and long-riffle pools in Long and Blaylock creeks.

Treatment	Mean width (m)	Mean length (m)	Mean area (m^2)	Mean depth (cm)	Riffle length (m)
Short riffle	6.4	27	181	52	8.6
Long riffle	6.3	32	198	55	51

Note: With the exception of riffle length, average habitat conditions of pools were statistically similar for both treatments (two-sample *t* tests, $P > 0.10$).

At each site, we collected fish by isolating the experimental pool with 6-mm-mesh block nets and sampling with a Smith–Root battery-powered backpack electrofisher. We typically sampled each pool for 30 min to 1 h or until no target species were collected on two consecutive passes. Fish were marked with treatment-specific caudal fin clips (i.e., long-riffle pools: upper caudal; short-riffle pools: lower caudal) and held overnight in a covered 2 × 2 m holding pen. We held fish because preliminary surveys indicated potentially high rates of emigration immediately after marking as a probable result of handling stress. In reviewing the methods of several movement studies, we discovered that this practice is not common. Typically, marked individuals are released immediately after being handled (e.g., Hill and Grossman 1987; Mundahl and Ingersoll 1989; Gatz and Adams 1994). While this approach may be acceptable in many situations (see Gowan and Fausch 1996), a recent study by Nordwall (1999) showed that electrofishing significantly increased the mobility of brown trout (*Salmo trutta*), especially within 24 h of handling (F. Nordwall, Department of Aquaculture, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden, personal communication). Because we allowed fish to recover from handling for approximately 24 h before removing the block nets, we felt that the risk of stress-related emigration was reduced, especially when compared with other movement studies.

The morning after marking, we released individuals from the holding pens but did not remove the block nets for 1 h. This allowed fish to move freely within the experimental pool and to reacclimate to pool conditions. Thirty minutes after removing the block nets, we initiated snorkeling surveys for marked fish in upstream and downstream pools immediately adjacent to the experimental pool. Simultaneous surveys of the two pools were completed hourly between 11:00 and 17:00 (six censuses) on day 0 and once per day between 11:00 and 14:00 on day 1 and day 3 for a total of eight censuses of marked fish. On day 1 and day 3, we expanded our snorkeling censuses to include the experimental pool and the second upstream pool and second downstream pool in the study reach. On day 0, we limited censuses of the experimental pool to the final survey (17:00) in order to minimize the risk that snorkeling might artificially inflate emigration.

We used visual census techniques rather than electrofishing, largely because our sampling design required repeated fish counts over short periods. We have shown previously that population estimates generated from snorkeling surveys in these streams are nearly identical to those generated from more invasive and labor-intensive electrofishing surveys (Lonzarich et al. 1998). High water clarity provided excellent conditions for identifying marked fish from distances up to 3 m.

An important assumption of our study was that marked fish observed in upstream and downstream pools accurately reflected emigration from experimental pools and was not biased downwards by fish emigrating from an experimental pool to become established in intervening riffles. Although we previously found strong differences in the pool and riffle assemblages of these streams (Lonzarich et al. 1998), we tested this assumption by tracking changes in the percentage of marked fish within experimental reaches. Our decision to accept or reject this assumption was based on an evaluation of the degree to which the total number of marked fish in long-riffle and short-riffle study reaches approximated the total number marked.

Statistics

We computed the proportion of emigrants (for each survey period) and the direction of emigration (either upstream or downstream) for all experimental pools. To test for treatment differences in emigration, we used two-sample *t* tests based on the maximum proportion of emigrants per pool (across all surveys). In long-riffle pools, the maximum number of known emigrants was typically observed on day 3, while in short-riffle pools, the maximum number was most often observed on day 1. Where proportion data deviated from normality, we performed square root arcsine transformations prior to analysis. We determined the directionality of movement by dividing the maximum number of marked fish that emigrated downstream by the combined number of emigrants. From this approach, values above 50% indicated a downstream bias in movement and below 50% indicated an upstream bias. To determine whether movement within a treatment was statistically biased in either direction, we employed the chi square goodness-of-fit test using the total number of upstream and downstream emigrants per treatment.

Results

General patterns of movement

We marked nearly 1800 individuals of the five target fish species, or an average of 147.7 (1 SE = ±19.7) individuals in each experimental pool (Table 2). Most marked fish were central stoneroller and striped shiner, and with the exception of striped shiner, we marked comparable numbers for each species in the two treatment categories. Our rates of resighting marked fish were high. For example, we observed an average of 70% (1 SE = ±3.5%) of the marked fish on the last hourly census of day 0 and 59% (1 SE = ±3.4%) on the last census (day 3).

We documented considerable movement of marked fish over the 3-day trial period. Overall, nearly one fifth (19%) of all marked individuals were observed in pools upstream or downstream of the experimental pools by day 3. Total movement by smallmouth bass (19%), central stoneroller (22%), and striped shiner (19%) were slightly higher than estimates for northern studfish (15%) and longear sunfish (13%). Overall movement was not directionally biased (52% of marked fish moved downstream and 48% moved upstream, Table 3). The only species showing any directional bias was smallmouth bass, which moved upstream twice as often as downstream (65 versus 35%, respectively; chi-square goodness-of-fit test, $P = 0.06$).

Treatment differences

Marked fish emigrated from short-riffle pools much more frequently (up to 10-fold) than from long-riffle pools. When examining emigration patterns on day 0, we found that 10%

Table 2. Total and average number of fish marked (per pool) from short-riffle and long-riffle pools in the two study streams.

Species	Short riffle	Long riffle	Average per pool
Central stoneroller	455	407	71.8
Striped shiner	177	363	45
Northern studfish	23	18	4.3
Longear sunfish	132	106	19.8
Smallmouth bass	42	43	7.1
Total	829	937	147.7

(1 SE = ±1.0%) of the marked fish in short-riffle pools had emigrated by the first survey. By comparison, no emigrants were observed from long-riffle pools until at least the third hour, and generally very few fish emigrated on day 0 (1.4%). This pattern held for the duration of the 3-day survey period. For all species combined, an average high of 29% (1 SE = ±1.5%) of marked fish were observed outside of experimental short-riffle pools whereas the average high for long-riffle pools was only 10% (1 SE = ±0.9%), or three times less than the percentage moving from short-riffle pools ($P < 0.01$). Because we confined snorkeling surveys to the two pools upstream and two pools downstream of the experimental pools, we consider these to be conservative estimates of movement.

In an effort to estimate the amount of movement beyond the study site boundaries, we graphed changes in the average percentage of marked fish within each study reach across the 3-day survey period (Fig. 2). These results show that the proportion of marked fish counted within long-riffle reaches did not change over time. In contrast, the percentage of marked fish declined sharply over time in short-riffle reaches from 67% for day 0 to 50% for day 3 (repeated measures ANOVA, $P = 0.02$). Assuming that all of these individuals (17%) moved beyond study reach boundaries by day 3, the actual percentage of fish emigrating from short-riffle pools appeared to be much higher than the average emigration of 29% that actually was observed.

To better describe the shape of the relationship between riffle length and emigration, we produced a multiple regression model using movement data collected for all 24 upstream and downstream riffles that were adjacent to the 12 experimental pools. Incorporating as factors both the potential emigrant pool (i.e., number tagged) and riffle length, our multiple regression model explained 60% of the variation in the emigration patterns of the target species. The model (emigrant number = 25.0 − 8.5 × ln riffle length + 0.09 × number tagged) showed that emigration was negatively affected by riffle length and to a much lesser degree positively correlated with the number of potential emigrants.

Fish from long-riffle pools not only emigrated less frequently, but they also showed a strong directional bias to movement that was not evident in short-riffle pools. Although marked fish from short-riffle pools moved upstream and downstream with equal frequency (chi-square goodness-of-fit test, $P = 0.70$, Table 3), emigrants from long-riffle pools moved downstream twice as often as they moved upstream (67 versus 33%; chi-square goodness-of-fit test, $P < 0.01$). This pattern held for three of the four species that moved from long-riffle pools. The exception was smallmouth bass, which showed a tendency to move upstream more often than downstream (chi-square goodness-of-fit test, $P = 0.07$).

Table 3. Percentage of marked fish that moved into sites downstream of experimental pools.

Species	All pools (%)	Long-riffle pools (%)	Short-riffle pools (%)
Central stoneroller	51	74**	45
Striped shiner	52	71**	46
Northern studfish	50	—	50
Longear sunfish	59	77[b]	45
Smallmouth bass	35[b]	23[b]	75[a]
Overall	52	67**	48

Note: Values were derived by dividing the number of marked fish downstream by the total number of emigrants. For each species, directional bias in movement was computed by chi-square goodness-of-fit test. *$P < 0.05$; **$P < 0.01$.

[a] Insufficient data for analysis.

[b] $P < 0.10$.

Treatment differences for individual species were highly variable, although there were strong similarities within families. Results for the two cyprinid species (central stoneroller and striped shiner) showed consistently high rates of movement from short-riffle pools (36 and 33%, respectively) and low rates from long-riffle pools (7 and 9%,) (two-sample t tests, $P < 0.01$). For northern studfish, we only observed movement from short-riffle pools (26%). By contrast, estimates of emigration for the two centrarchids revealed no general pattern. Smallmouth bass moved more often from long-riffle pools (28 versus 10%), although this difference was only weakly significant (two-sample t test, $P = 0.09$), whereas longear sunfish emigrated with equal frequency from pools in the two treatments. We note, however, that the average movement by longear sunfish from long-riffle pools was affected strongly by results from one site. When we removed the data from this site, the average for this species declined more than 50% (13 to 6%). This new estimate was slightly lower than the average movement from short-riffle pools (14%, two-sample t test, $P = 0.07$).

Discussion

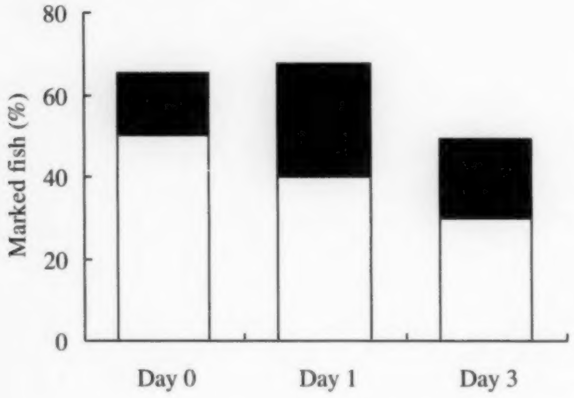
General patterns of movement

Based on observations encompassing nearly 1800 marked fish, movement patterns within our two study streams could be generally described as a diffusive process, with target species spreading equally in upstream and downstream directions from experimental pools.

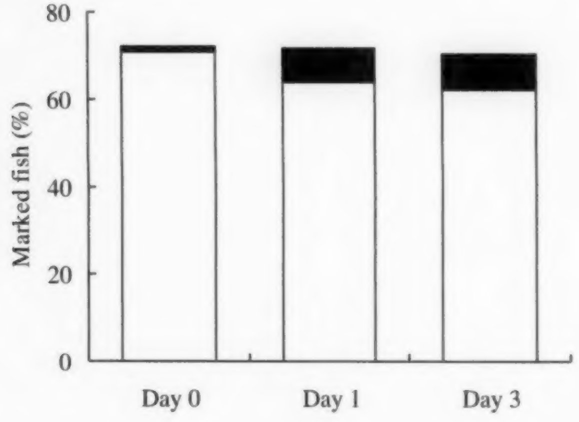
Even if we exclude individuals that moved beyond the study area boundaries, the levels of movement documented here are high when compared with results from similar studies. Minimally, one fifth of all marked fish emigrated from experimental pools, moving 30–100 m into neighboring habitats, over the 3-day trials. These numbers are remarkable, given that most studies report significantly less movement over much longer periods of time. For example, in an 18-month study, Hill and Grossman (1987) estimated that the home range size of three small warmwater fish species was less than 20 m. Specific home range estimates for smallmouth bass and longear sunfish are slightly higher,

Fig. 2. Percentage of marked fish moving from (black bars) and remaining within (white bars) (*a*) short-riffle pools and (*b*) long-riffle pools on each day of the 3-day survey period. Only in the short-riffle treatment did daily percentages of fish observed in experimental pools decline significantly over time (repeated measures ANOVA, $P = 0.02$). Percent emigrants for day 0 reflect data for marked fish observed one pool upstream and one pool downstream of the experimental pool. For day 1 and day 3, these percentages were based on marked fish observed two pools upstream and two pools downstream.

a) Short-Riffle Pools



b) Long-Riffle Pools



ranging from 30 to 100 m (Gerking 1953; Berra and Gunning 1972). Mundahl and Ingersoll (1989) estimated that central stoneroller moved an average of only 35 m in 1 month.

Although we cannot estimate maximum movement distances for our marked fish, our results do not support the long-held view of restricted fish movement first proposed by Gerking (1953, 1959). Since his seminal work, research on fish movement has overwhelmingly supported the idea that stream fishes are restricted to relatively small areas (see review by Gowan et al. 1994). This view of spatially discrete populations has important biological implications. For example, it forms the basis for the strong species–habitat relationships often found in streams (e.g., Gorman and Karr 1978). Moreover, habitat and species management decisions are by necessity based on information concerning the home range size of target species.

Funk (1955) and Gowan et al. (1994) presented strong challenges to the restricted movement model. In his study of fishes in Missouri streams, Funk (1955) recorded extensive movement by 14 species (up to 40 km) and concluded that stream fish populations consist of both mobile and sedentary individuals. More recently, Gowan et al. (1994) measured extensive movement in stream-dwelling brook trout (*Salvelinus fontinalis*) and argued that field studies might be seriously biased against detecting movement. They were particularily critical of the cavalier dismissal of characteristically low recapture rates (<50%) in data analyses of traditional movement studies. They claimed, and we agree, that low recapture rates may in fact represent the movement of fishes beyond study area boundaries. Supporting the view of extensive fish movement are studies on fish recolonization that measure movement indirectly. These studies have universally shown very rapid (i.e., hours to months) movement into defaunated reaches and habitat-units (e.g., Matthews 1986; Sheldon and Meffe 1995; Lonzarich et al. 1998). Recently, Warren and Pardew (1998) found retention rates within 36-m segments of <30% of marked fishes, suggesting up to a 70% emigration rate over a 17-day period. Smithson and Johnston (1999) also measured movement as high as 33% for fishes in a nearby Arkansas stream.

Effects of riffle length on movement

Our results indicate a very strong relationship between the proximity of neighboring stream pools (as determined by intervening riffle lengths) and daily interhabitat movements of fishes. These findings are consistent with a previous study where we found that long riffles significantly slowed fish recolonization to defaunated pools (Lonzarich et al. 1998). Schaefer (1999) also reported an effect of riffle length on movement in an artificial stream channel; however, ours is the first study to quantify the degree to which riffles restrict short-term fish movement between patches in natural streams.

In some respects, these findings are not surprising, given that ecologists have long recognized the effects of habitat isolation, or interpatch distance, on the movement of organisms between equivalent habitat patches (e.g., Forman and Godron 1986; Forman 1995). As is true for organisms in other spatially heterogeneous environments, fish in streams are distributed within a mosaic of habitats of varying quality (e.g., food availability, predator densities, physical conditions). Under these conditions, fish may make frequent explorations of different habitats (e.g., pools) in search of food resources (Fraser and Sise 1980). However, as indicated by our results, long riffle corridors can limit these excursions by pool-dwelling species and therefore may reduce the ability of individuals to evaluate differences in resource conditions (e.g., food, predators) across pools. Matthews (1998) also speculated that shallow riffle corridors, acting as filters to the movement of small-bodied prey and large-bodied predators, could affect predator–prey relationships in stream reaches.

As suggested by Matthews (1998), water depth probably contributes significantly to the restricted movement of fish through riffle corridors, possibly due to the fact that shallow waters expose fish to terrestrial predators (e.g., Power 1984, 1987). We would add water currents as a factor as well, especially for long riffles, where strong currents may prove difficult to negotiate. Although direct tests of these potential factors are lacking, Warren and Pardew (1998) measured strong effects of water current on fish movement across shallow-water road crossings, and we found that fish from long-riffle pools moved significantly less frequently against the current (upstream) than with the current (downstream). We also have measured a negative effect of water depth on fish recolonization to defaunated stream pools (Lonzarich et al. 1998).

While we suspect that these factors are important determinants of fish movement through long riffles, we are careful not to extrapolate beyond the temporal scope (i.e., hours or days) and low-flow conditions that characterized our study. In fact, we question whether potential survival or energy costs associated with traversing long riffles affect patterns of fish redistribution during floods or seasonal migrations to spawning or wintering habitats. Still, the timing of this study (the summer) coincided with a critical period in the life cycle of stream fish species. Not only is this the season when fish experience their most favorable growing conditions, it is also when competition and threats of predation, especially for juveniles, can be very intense.

The potential effects of pool isolation that we envision are derived from two streams with moderately high pool to riffle ratios. We would not expect any habitat isolation in sandy bottom streams that lack the physical conditions necessary to promote riffle development. However, we would predict even more severe effects of pool isolation in small, high-gradient, and gravel-bottom mountainous streams where neighboring pools can be isolated by long stretches of shallow water and high-gradient riffles (e.g., Beechie and Sibley 1997).

In conclusion, we believe that our results add significantly to our understanding of how spatial variability in habitats affects the distribution and dynamics of fish populations and assemblages in streams. Moreover, because land-use activities can change channel characteristics and alter the spacing of stream habitats, our findings may aid in efforts to better predict the ecological consequences of such activities.

Acknowledgements

We are grateful to the Ouachita National Forest for granting access to Long and Blaylock creeks, to W. Matthews for providing advice and support in the development of the study, and to J. Guldin for administrative support. We are deeply indebted to both A. Clingenpeel and B. Crump for logistical assistance while we were in the field. The work was funded by the USDA Forest Service, Ouachita Mountain Ecosystem Management Research Project, Southern Research Station.

References

Beechie, T.J., and Sibley, T.H. 1997. Relationships between channel characteristics, woody debris and fish habitat in northwestern Washington streams. Trans. Am. Fish. Soc. **126**: 217–229.

Berra, T.M., and Gunning, G.E. 1972. Seasonal movement and home range of the longear sunfish, *Lepomis megalotis* (Rafinesque) in Louisiana. Am. Midl. Nat. **88**: 368–375.

Clingenpeel, J.A. 1994. A cumulative effects analysis of silvicultural best management practices using Basin Area Stream Survey (BASS) methods. USDA Forest Service, Southern Region, Ouachita National Forest, Hot Springs, Ark.

Congdon, B.C. 1995. Unidirectional gene flow and maintenance of genetic diversity in mosquitofish *Gambusia holbrooki* (Teleostei: Poeciliidae). Copeia, **1995**: 162–172.

Fausch, K.D., and Young, M.K. 1995. Evolutionary significant units and movement of resident stream fishes: a cautionary tale. Am. Fish. Soc. Symp. **17**: 360–370.

Forman, R.T. 1995. Land mosaics: the ecology of landscapes and regions. Cambridge University Press, Cambridge, U.K.

Forman, R.T., and Godron, M. 1986. Landscape ecology. John Wiley & Sons, New York.

Fraser, D.F., and Sise, T.E. 1980. Observations on stream minnows in a patchy environment: a test of a theory of habitat distribution. Ecology, **61**: 790–797.

Freeman, M.C. 1995. Movements by two small fishes in a large stream. Copeia, **1995**: 361–367.

Funk, J.L. 1955. Movement of stream fishes in Missouri. Trans. Am. Fish. Soc. **85**: 39–57.

Gatz, A.J., and Adams, S.M. 1994. Patterns of movment of centrarchids in two warmwater streams. Ecol. Freshwater Fish, **3**: 35–48.

Gerking, S.D. 1953. Evidence for the concepts of home range and territory in stream fishes. Ecology, **34**: 347–365.

Gerking, S.D. 1959. The restricted movement of fish populations. Biol. Rev. Camb. Philos. Soc. **34**: 221–242.

Gorman, O.T., and Karr, J.R. 1978. Habitat structure and stream fish communities. Ecology, **59**: 507–515.

Gowan, C., and Fausch, K.D. 1996. Mobile brook trout in two high-elevation Colorado streams: re-evaluating the concept of restricted movement. Can. J. Fish. Aquat. Sci. **53**: 1370–1381.

Gowan, C., Young, M.K., Fausch, K.D., and Riley, S.C. 1994. Restricted movement in resident stream salmonids: a paradigm lost? Can. J. Fish. Aquat. Sci. **51**: 2626–2637.

Hill, J.H., and Grossman, G.D. 1987. Home range estimates of North American stream fishes. Copeia, **1987**: 19–25.

Karr, J.R., and Schlosser, I.J. 1978. Water resources and the landwater interface. Science (Washington, D.C.), **201**: 229–234.

Keller, E.A., and Swanson, F.J. 1979. Effects of large organic material on channel form and fluvial processes. Earth Surf. Processes, **4**: 361–380.

Kruse, C.G., Hubert, W.A., and Rahel, F.J. 1997. Geomorphic influences on the distribution of Yellowstone cutthroat trout in the Absaroka Mountains, Wyoming. Trans. Am. Fish. Soc. **126**: 418–427.

Lonzarich, D.G., Warren, M.L., Jr., and Elger-Lonzarich, M.R. 1998. Effects of habitat isolation on the recovery of fish assemblages in experimentally defaunated stream pools in Arkansas. Can. J. Fish. Aquat. Sci. **55**: 2141–2149.

Matthews, W.J. 1986. Fish faunal structure in an Ozark stream: stability, persistence and a catastrophic flood. Copeia, **1986**: 388–397.

Matthews, W.J. 1998. Patterns in freshwater fish ecology. Chapman and Hall, New York.

Matthews, W.J., Harvey, B.C., and Power, M.E. 1994. Spatial and temporal patterns in the fish assemblages of individual pools in a midwestern stream (USA). Environ. Biol. Fishes, **39**: 381–397.

Mundahl, N.D., and Ingersoll, C.G. 1989. Home range, movements, and density of the central stoneroller, *Campostoma* *anomalum*, in a small Ohio stream. Environ. Biol. Fishes, **24**: 307–311.

Nordwall, F. 1999. Movements of brown trout in a small stream: effects of electrofishing and consequences for population estimates. N. Am. J. Fish. Manage. **19**: 462–469.

Power, M.E. 1984. Depth distributions of armored catfish: predator-induced avoidance? Ecology, **65**: 523–528.

Power, M.E. 1987. Predator avoidance by grazing fishes in temperate and tropical streams: importance of stream depth and prey size. *In* Predation: direct and indirect impacts on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Hanover, N.H. pp. 333–352.

Ralph, S.C., Poole, G.C., Conquest, L.L., and Naiman, R.J. 1994. Stream channel morphology and woody debris in logged and unlogged basins in western Washington. Can. J. Fish. Aquat. Sci. **51**: 37–51.

Schaefer, J.F. 1999. Movement by three stream-dwelling cyprinids (*Notropis boops*, *Campostoma anomalum*, *Cyprinella venusta*). Ph.D. dissertation, University of Oklahoma, Norman, Okla.

Schlosser, I.J. 1991. Stream fish ecology: a landscape perspective. BioScience, **41**: 704–712.

Schlosser, I.J. 1995. Critical landscape attributes that influence fish population dynamics in headwater streams. Hydrobiologia, **303**: 71–81.

Sheldon, A.L., and Meffe, G.K. 1995. Short-term recolonization by fishes of experimentally defaunated pools of a coastal plain stream. Copeia, **1995**: 828–837.

Smithson, E.B., and Johnston, C.E. 1999. Movement patterns of stream fishes in a Ouachita highlands stream: an examination of the restricted movement paradigm. Trans. Am. Fish. Soc. **128**: 847–853.

Taylor, C.M. 1997. Fish species richness and incidence patterns in isolated and connected stream pools: effects of pool volume and spatial position. Oecologia, **110**: 561–566.

Ward, J.V. 1998. Riverine landscapes: biodiversity patterns, disturbance regimes and aquatic conservation. Biol. Conserv. **83**: 269–278.

Warren, M.L., Jr., and Pardew, M.G. 1998. Road crossings as barriers to small-stream fish movement. Trans. Am. Fish. Soc. **127**: 637–644.

Current, reconstructed past, and projected future status of brook trout (*Salvelinus fontinalis*) streams in Virginia

Arthur J. Bulger, Bernard J. Cosby, and James R. Webb

Abstract: Southern Appalachian streams host a rich diversity of fishes, but the Southern Appalachian Assessment concluded that 70% of stream locations showed significant fish community degradation, partly due to acid deposition. About 40% of total Southern Appalachian trout stream length occurs in Virginia. Our research in Shenandoah National Park, Virginia, has documented both chronic and episodic acidification in streams and brook trout (*Salvelinus fontinalis*) mortality during acid episodes. Here, we analyze a representative sample of 60 Virginia brook trout streams on noncarbonate bedrock with minimal human disturbance in their watersheds. Of more than 300 such streams in Virginia, only about 50% are currently suitable for brook trout, based on their acidification status. For the population of brook trout streams to which this analysis applies, model-based projections indicate that continued sulfate deposition at 1991 levels will result in about 70 additional streams becoming chronically acidic and unsuitable for brook trout. A 40% reduction will result in about 48 additional chronically acidic streams. Even a 70% reduction will likely result in about 15 additional streams becoming acidified. We conclude that reductions in sulfate deposition greater than 70% (relative to 1991 levels) are needed to prevent more brook trout stream losses in Virginia.

Résumé : Les cours d'eau du sud des Appalaches abritent une faune ichtyenne très diversifiée, mais l'évaluation qui en a été faite a conclu que 70% des cours d'eau présentaient une dégradation marquée des communautés de poissons, phénomène dû en partie aux retombées acides. On retrouve en Virginie environ 40% de l'ensemble des rivières à truite du sud des Appalaches. Notre recherche, menée dans le parc national Shenandoah, en Virginie, a fait ressortir l'existence d'une acidification à la fois chronique et épisodique des cours d'eau, les épisodes d'acidification causant une mortalité chez l'omble de fontaine (*Salvelinus fontinalis*). Nous analysons ici un échantillon représentatif de 60 rivières à omble de fontaine de Virginie, à assise rocheuse non carbonatée, dont les bassins présentent un minimum de perturbation anthropique. Sur plus de 300 cours d'eau de ce type en Virginie, seuls ~50% offrent un habitat convenable à l'omble de fontaine, selon leur niveau d'acidification. Pour le groupe de rivières à omble de fontaine auquel s'applique cette analyse, les projections après modélisation indiquent qu'une poursuite des dépôts de sulfate au niveau de 1991 va causer l'acidification chronique de ~70 cours d'eau de plus, qui deviendront impropres à la vie de l'omble de fontaine. Une réduction de 40% ferait que ~48 rivières de plus deviendraient chroniquement acidifiées. Même une réduction de 70% causerait vraisemblablement l'acidification de ~15 cours d'eau supplémentaires. Nous concluons que les réductions des dépôts de sulfate doivent être supérieurs à 70% (par rapport aux niveaux de 1991) si l'on veut empêcher la perte de rivières à omble de fontaine en Virginie.

[Traduit par la Rédaction]

Introduction

Permanent water bodies are conspicuous features in the Southern Appalachian Mountains. The mean density of streams and river channels is more than 9 m of length per hectare of land, and river and lake surface is at least 1.5% of the total surface area of the Southern Appalachians. Residents regard water bodies as extremely important, and multiple uses include drinking water, transportation, agriculture, flood control, hydroelectric power, wildlife observation, waterfront human habitation, and fishing and other aquatic recreation (Southern Appalachian Man and the Biosphere (SAMAB) 1996). Fish diversity in the Southern Appalachians is high. Of the approximately 950 species of freshwater fish in North America, about 485 species are found in the southeast (Jenkins and Burkhead 1993; Walsh et al. 1995). The total numbers of freshwater fish species per state in the region range from 107 in Maryland to 307 in Tennessee (Jenkins and Burkhead 1993).

A regional survey conducted as part of the Southern Appalachian Assessment (SAA) (SAMAB 1996) concluded that 70% of sampled locations on streams show moderate to severe fish community degradation and that about 50% of the stream length in West Virginia and Virginia shows structural habitat impairment.

The Mid-Atlantic Highlands has one of the highest rates of acid deposition in the United States (Herlihy et al. 1993, 1996). The region most at risk is located along the Appalachian Mountain chain from the Adirondacks in New York to

Received October 8, 1998. Accepted March 22, 2000.
J14823

A.J. Bulger,[1] B.J. Cosby, and J.R. Webb. Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22903, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: abulger@Virginia.edu

the Southern Blue Ridge in Georgia (National Acid Precipitation Assessment Program (NAPAP) 1990). There have been significant historical declines in the acid-neutralizing capacity (ANC) of stream waters in the region as a result of chronic acid deposition (Kaufmann et al. 1988). Acidification is a conspicuous threat to three trout species in the region: brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*) (reproducing populations of any of the three are often referred to as "wild" trout). Of the three, native brook trout is the most acid tolerant, brown trout introduced from Europe is intermediate in acid tolerance, and rainbow trout introduced from the western United States is most sensitive (Gagen and Sharpe 1987; Freda et al. 1991). The Fish in Sensitive Habitats Project (Bulger et al. 1995, 1999; SAMAB 1996) demonstrated that both chronic and episodic acidification occurs in streams in the Shenandoah National Park in Virginia, lowering fish species richness and fish condition factor and causing mortality of brook trout during acid episodes. Data from the Great Smoky Mountains National Park in Tennessee and North Carolina indicate poor buffering capacity for most streams and low pH at higher elevations. The fish and aquatic insects of the St. Mary's River in Virginia were surveyed in the 1930s and again in 1988. Significant loss of biodiversity from acidification effects was apparent (Mohn et al. 1989).

Approximately 40% (6×10^6 ha) of the area of the Southern Appalachian Mountains is in the range of brook, rainbow, and (or) brown trout with up to 53 000 km of potential trout streams (SAMAB 1996). Virginia alone accounts for 39% of regional trout stream length, with 32% in North Carolina, 10% in Georgia, 10% in Tennessee, 7% in West Virginia, 2% in South Carolina, and 0% in Alabama. The SAA concluded that about 27% of all trout stream length in the Southern Appalachian mountains is in landscapes that are moderately vulnerable to acidification, and approximately 59% is in areas that are highly vulnerable to acidification (SAMAB 1996). Our objectives in this study were to assess the current status, reconstruct the past status, and project the future responses to acidic deposition of brook trout streams in Virginia. We focus on brook trout because it is the most widespread of the three trout species, the only native trout species, and thus the only trout species for which a reconstructed past status is meaningful. Within Virginia, one or more of the three trout species are found in 3265 river kilometres; of these, 2198 river kilometres (67%) harbor only brook trout, more than in the other southeastern states combined (Jenkins and Burkhead 1993).

Methods

Our analysis of current, reconstructed, and projected future status of brook trout streams in Virginia requires three components: (*i*) a scheme for categorizing brook trout status in streams based on stream water quality, (*ii*) observations of current water quality in a regionally representative sample of streams in Virginia (to establish current conditions and to calibrate the model), and (*iii*) a catchment biogeochemical model for forecasting and reconstructing water quality for the streams (and the additional data necessary for model calibration and implementation: atmospheric deposition, soil chemistry, etc.).

Brook trout status related to water quality

We use four categories of brook trout status defined by the annual (volume-weighted) average value of stream ANC. The ANC ranges of the four categories are chosen to represent thresholds for responses of brook trout to acidic conditions in streams (Table 1 and below). This classification scheme was a principal result of the Fish in Sensitive Habitats study, a 3-year field experiment studying the responses of fish (individuals, populations, and communities) to changes in acid–base status in streams in the Shenandoah National Park (Bulger et al. 1999, Vol. IV, p. 108). Implicit in the scheme is the assumption that habitat in the streams is otherwise appropriate for brook trout, which was accounted for in that study.

Streams that are *not acidic* (annual average ANC >50 μequiv.$\cdot L^{-1}$) are generally *suitable* for brook trout because they have a large enough buffering capacity that persistent acidification poses no threat to brook trout and there is little likelihood of storm-induced acid episodes lethal to brook trout. The ANC in these streams typically remains above zero in all seasons and flow regimes. As a result, reproducing brook trout populations are expected. ANC values below 200 μequiv.$\cdot L^{-1}$ have been regarded elsewhere as "sensitive" to acidification for general ecological purposes (Altshuller and Lindhurst 1984; Winger et al. 1987; Knapp et al. 1988). Bulger et al. (1998), examining brook trout response only, set the threshold for unaffected streams much lower because brook trout are a relatively acid-tolerant species.

Streams that we term *indeterminate* (annual average ANC from 20 to 50 μequiv.$\cdot L^{-1}$) comprise a problematic category for prediction of both acid deposition effects and brook trout status. Streams in this category have been designated elsewhere as "extremely sensitive" to acidification (Gibson et al. 1983; Schindler 1988) because any further reduction in ANC can produce extremely deleterious biological effects. In this range of average annual ANC, streams may or may not experience lethal episodic acidification during storms. The occurrence of episodic acidity depends on a number of hydrologic, physical, and chemical characteristics that cannot be readily predicted, such as the ratio of stormflow to baseflow (a function of the geomorphology of the catchment and storm severity) and the occurrence of springs or minor alkaline tributaries (that can buffer storm events at these levels of ANC and (or) provide refugia). The status of brook trout populations is also difficult to predict in these streams. Brook trout populations can be healthy if other habitat characteristics are favorable or poor if the habitat quality is also poor. In general, membership in this category can be regarded as transient, as streams shift between the not acidic and episodically acidic categories (and brook trout status shifts between suitable and marginal) during acidification and recovery from acidification.

Streams that are *episodically acidic* (annual average ANC from 0 to 20 μequiv.$\cdot L^{-1}$) are *marginal* for brook trout because they have so little buffering capacity that acid episodes are likely to occur (Hyer et al. 1995). Although the frequency and magnitude of episodes vary, streams in this category have likely already lost sensitive fish species and display a reduced species richness. Examples of species lost might include longnose dace (*Rhinichthys cataractae*), mottled sculpin (*Cottus bairdi*), northern hog sucker (*Hypentelium nigricans*), central stoneroller (*Campostoma anomalum*), river chub (*Nocomis micropogon*), and rosyside dace (*Clinostomus funduloides*) (Heard et al. 1997; Bulger et al. 1999). There are measurable sublethal stresses on individuals in these streams, including low body weight and condition factor in blacknose dace (*Rhinichthys atratulus*) and brook trout (Dennis and Bulger 1995; Dennis et al. 1995; Bulger et al. 1998) and lower population density in brook trout (Bulger et al. 1998) and other more acid-tolerant species (Bulger et al. 1995). Missing year-classes of brook trout also become more likely. Streams in this category can have baseflow chemistry tolerable to brook trout fry but acidic episodes lethal to fry (MacAvoy and Bulger 1995).

Table 1. Brook trout stream categories, ANC classes, and ANC thresholds for brook trout responses to acidification in forested headwater catchments in western Virginia (Bulger et al. 1999).

Brook trout stream category	ANC class	ANC range (μequiv.$\cdot$L^{-1})	Brook trout response
Suitable	Not acidic	>50	Reproducing brook trout populations expected where habitat suitable
Indeterminate	Indeterminate	20–50	Extremely sensitive to acidification; brook trout response variable
Marginal	Episodically acidic	0–20	Sublethal and (or) lethal effects on brook trout possible
Unsuitable	Chronically acidic	<0	Lethal effects on brook trout probable

Streams that are *chronically acidic* (annual average ANC < 0 μequiv.$\cdot$L^{-1}) are *unsuitable* for brook trout. Forested headwater streams in the mountains of Virginia with annual average ANCs less than zero have negative ANCs for most of the year, not just during storm events. As a result, the biological communities of streams in this category are impoverished. Loss of even acid-tolerant species occurs in these streams, and species richness is very low (Bulger et al. 1999). Such streams cannot support healthy brook trout populations.

Stream water quality data

In order to evaluate the current status of brook trout streams in western Virginia and to calibrate the biogeochemical model used for projecting the past and future brook trout status of the streams, we needed regionally representative water quality data that spanned a number of years. We obtained such data from two closely coordinated research and monitoring programs at the University of Virginia that focus on the biogeochemistry of forested mountain watersheds in western Virginia: the Virginia Trout Stream Sensitivity Study (VTSSS) and the Shenandoah Watershed Study (SWAS). The VTSSS program was initiated in the spring of 1987. An initial survey was conducted in which stream water quality was sampled for 344, or about 78%, of the state's identified native brook trout streams (Fig. 1). The streams not included in the survey were those known to have substantial direct human disturbance in the watershed. Analysis of the survey data disclosed a strong correlation between local geology and the acid–base status of the streams (Webb et al. 1994). The surveyed streams were subsequently stratified by geology and a representative subset selected for long-term water quality monitoring. The VTSSS program presently includes 55 streams, mostly located on National Forest lands, which are sampled on a quarterly basis. The SWAS program was initiated in 1979. Weekly stream water sampling was begun on two streams in Shenandoah National Park as part of a study of biogeochemical cycling within forested watersheds and their responses to anthropogenic perturbations. The SWAS program presently includes six streams that are sampled on a weekly basis and nine streams that are sampled on a quarterly basis. The SWAS quarterly sampling is scheduled to coincide with VTSSS quarterly sampling. In both programs, quarterly stream samples are taken during the last weeks of January, April, July, and October.

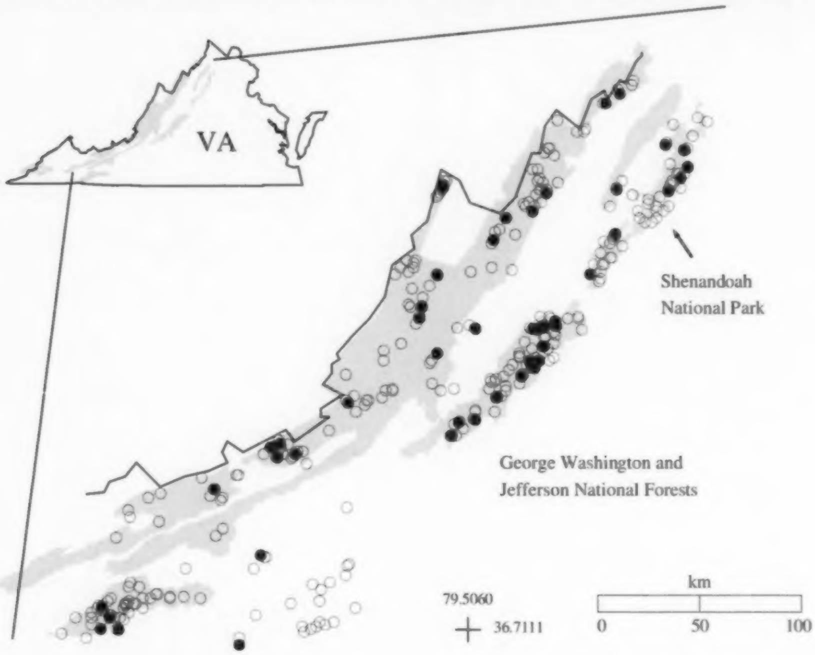
The two programs produced continuous (no missing samples) quarterly water quality data for 70 sites in western Virginia for the period 1989–1993. Ten of these sites have carbonate as the dominant bedrock type in their catchments and were excluded because these streams are not considered susceptible to acidification. We used the remaining 60 streams for this analysis of current, past, and potential future brook trout status in the streams of western Virginia. Although the exact total of native brook trout streams in Virginia is uncertain, the Virginia Department of Game and Inland Fisheries has estimated that approximately 440 streams in Virginia originally harbored reproducing populations of brook trout (Jenkins and Burkhead 1993). Of these, approximately 20% have substantial direct human disturbance in their watershed (housing, agriculture, etc.) and were therefore excluded from the VTSSS and SWAS (and therefore from this analysis). An additional approximately 10% of the total population of brook trout streams identified by the Virginia Department of Game and Inland Fisheries have extensive carbonate bedrock in their watersheds. We do not consider these streams in this analysis because they are not considered susceptible to acidification. Therefore, the subsample of 60 streams used here can be considered to represent approximately 70% of the state's historical trout streams (the population of brook trout streams in Virginia that do not lie primarily on limestone and (or) have not been significantly affected by human activity within the watersheds). We will use these 60 streams for which we have detailed information to draw inferences about the entire population of slightly more than 300 brook trout streams in Virginia that are at risk from acid deposition.

Stream water samples collected for the VTSSS and SWAS programs were analyzed for sulfate, nitrate, chloride, hydrogen ion, calcium, magnesium, potassium, sodium, ANC, and pH. Dissolved organic carbon (and thus organic acidity) in these streams is negligible (<1.0 mg$\cdot$L^{-1}). Details of analytical and quality assurance methods are posted on the SWAS–VTSSS website at http://wsrv.clas.virginia.edu/~swasftp. For this assessment, we defined the ANC as the sum of base cation concentrations (calcium, magnesium, potassium, and sodium) minus the sum of acid anion concentrations (sulfate, nitrate, and chloride), with concentrations expressed as microequivalents per litre. We calculated discharge-weighted annual average concentrations from the quarterly sample concentrations for use in the model calibrations. Measured stream discharge is available only for the six streams monitored on a weekly basis. We therefore estimated one set of quarterly discharge weights for all 60 sites by identifying 35 U.S. Geological Survey stream gauging stations in western Virginia that have locations and elevations similar to those of the quarterly sampling sites. Average quarterly stream discharges were determined for each station from 1985 to 1994 (with the end of each discharge quarter coinciding with the quarterly stream sampling times). The average quarterly discharges were normalized to the annual discharge at each site. The normalized quarterly discharges were averaged across all sites to provide the regional quarterly discharge weights.

Model of water quality response to acidic deposition

MAGIC (model of acidification of groundwater in catchments) (Cosby et al. 1985*a*, 1985*b*, 1985*c*) was the principal model used by the NAPAP to estimate future damage to lakes and streams in the eastern United States (Thornton et al. 1990; NAPAP 1991). The validity of the model has been confirmed by comparison with estimates of lake acidification inferred from paleolimnological reconstructions of historical lake changes in pH (Sullivan et al. 1991, 1996) and with the results of several catchment-scale acidification and deacidification experiments (e.g., Cosby et al. 1995, 1996).

Fig. 1. Locations of the 344 brook trout streams in Virginia that were sampled as part of the 1987 VTSSS survey. The solid circles identify the VTSSS streams that are being monitored on a quarterly basis. Shaded areas are National Park and National Forest Lands.



MAGIC has been used to reconstruct the history of acidification and to simulate the future trends on a regional basis and in a large number of individual catchments in both North America and Europe (e.g., Jenkins et al. 1990; Norton et al. 1992; Wright et al. 1994; Cosby et al. 1996).

MAGIC is a lumped-parameter model of intermediate complexity, developed to predict the long-term effects of acidic deposition on surface water chemistry. The model simulates soil solution chemistry and surface water chemistry to predict the monthly and annual average concentrations of the major ions in these waters. MAGIC consists of (*i*) a section in which the concentrations of major ions are assumed to be governed by simultaneous reactions involving sulfate adsorption, cation exchange, dissolution–precipitation–speciation of aluminum, dissolution–speciation of inorganic carbon, and dissociation of organic acids and (*ii*) a mass-balance section in which the flux of major ions to and from the soil is assumed to be controlled by atmospheric inputs, chemical weathering, net uptake and loss in biomass, and losses to runoff. As implemented in this project, the model is a two-compartment representation of a catchment. Atmospheric deposition enters the soil compartment and the equilibrium equations are used to calculate soil water chemistry. The water is then routed to the stream compartment and the appropriate equilibrium equations are reapplied to calculate stream water chemistry. At the heart of MAGIC is the size of the pool of exchangeable base cations in the soil. As the fluxes to and from this pool change over time owing to changes in atmospheric deposition, the chemical equilibria between soil and soil solution shift to give changes in surface water chemistry. The degree and rate of change of surface water acidity thus depend both on flux factors and on the inherent characteristics of the affected soils.

Calibration of the model

The aggregated nature of the model requires that it be calibrated to observed data from a system before it can be used to examine potential system response. Calibration is achieved by setting the values of certain parameters within the model that can be directly measured or observed in the system of interest (fixed parameters, e.g., soil physical characteristics). The model is then run (using observed atmospheric and hydrologic inputs) and the outputs (stream water and soil chemical variables) are compared with observed values of these variables. If the observed and simulated values differ, the values of another set of parameters in the model (optimized parameters, e.g., cation weathering rates) are adjusted to improve the fit. After a number of iterations, the simulated minus observed values of the criterion variables usually converge to zero (within some specified tolerance). The model is then considered calibrated.

Calibrations are based on volume-weighted mean annual fluxes for a given period of observation. The length of the period of observation used is not arbitrary. Model output will be more reliable if the annual flux estimates used in calibration are based on a number of years rather than just 1 year. Averaging over a number of years reduces the likelihood that an "outlier" year (very dry, etc.) is the primary data on which model forecasts are based. On the other hand, averaging over too long a period may remove important trends in the data that need to be simulated by the model. For this study, the model was calibrated using 5 years of data (1989–1993 calendar years) to provide the volume-weighted annual flux estimates for both deposition and runoff. The calibration procedure requires that stream water quality, soil chemical and physical characteristics, and atmospheric deposition data be available for each stream. The water quality data have been discussed above. There are no direct measurements of either atmospheric deposition or soil properties at each of the 60 streams for which water quality data are available. We estimated (see following paragraphs) the requisite data for each site by (*i*) assigning soil properties based on landscape classification of each stream's catchment and (*ii*) using model extrapolations from a national atmospheric deposition monitoring network.

Landscape classes and soils data

The forested mountain watersheds that provide habitat for brook trout in western Virginia are more sensitive to the effects of acidic deposition than other landscape types in the Southern Appalachian region (valley floors, agricultural lands, etc.). Among forested mountain watersheds, however, there is a still a range of sensitivity to acidic deposition, correlated with local geology, that can provide a further stratification of the landscape. This stratification provides the landscape classes needed to associate soil properties with each of the selected streams (a prerequisite for model calibration). The assignment of each of the study streams to a landscape class also allows extrapolation of results from the study streams to the entire regional population of trout streams. Webb et al. (1994) identified six geological classes that account for much of the spatial variation in ANC among the VTSSS and SWAS quarterly sampling sites. We reduced the six classes described by Webb et al. (1994) to four classes for this analysis. Streams in the carbonate class were not included. We defined a single siliciclastic class for the Valley and Ridge Province that includes both the "siliciclastic" and "minor carbonate" classes (the minor carbonate catchments are actually predominantly siliciclastic with minor carbonate inclusions). The landscape classes adopted for this study and the numbers of selected stream sites within each of the classes are thus Blue Ridge siliciclastic (16 streams), Blue Ridge granitic (18 streams), Blue Ridge basaltic (four streams), and Valley and Ridge siliciclastic (22 streams).

We derived regional estimates of stream responses using a weighting scheme based on the number of modelled streams in each of the bedrock classes and the total number of VTSSS streams in each of these bedrock classes. For instance, 103 of the VTSSS streams occur on granitic bedrock and 18 of those streams were modelled and used for hindcasts and forecasts. A weight of 5.7 (=103/18) is applied to the results of each of the 18 streams in this bedrock class when statistical summaries are produced. The weights for streams in the other bedrock classes are 6.25 (=25/4) for basaltic, 4.3 (=69/16) for Blue Ridge siliciclastic, and 4.86 (=107/22) for Valley and Ridge siliciclastic. Thus, in presenting results when we refer to percentages of streams having a certain brook trout status, we are not referring to percentages of the 60 streams actually calibrated but rather to percentages of the entire regional population of brook trout streams considered in this study. That regional population of brook trout streams consists of all brook trout streams in Virginia that do not lie primarily on limestone and (or) have not been significantly affected by human activity within the watersheds (approximately 310 streams), which, in turn, is 70% of the total population of brook trout streams identified by the Virginia Department of Game and Inland Fisheries in Virginia (approximately 440 streams; see above).

Soils data for use in the model application were available for 18 sampling pits located in five forested mountain watersheds in western Virginia. The data for four of the watersheds were obtained in 1986 and 1987 through the Direct-Delayed Response Program (DDRP), a component of the NAPAP. The methods of soil sampling and analysis used for the DDRP are described in Church et al. (1992). The data for the fifth watershed were obtained through the SWAS program using methods closely comparable with those of the DDRP. The soils data for the individual soil horizons at each sampling pit were aggregated based on horizon, depth, and bulk density to obtain single vertically aggregated values for each pit. The soil parameters used in the model included soil depth, bulk density, pH, cation-exchange capacity, and exchangeable bases on the soil (calcium, magnesium, potassium, and sodium). The soils data were assigned to each of the geologically defined landscape classes depending on the type of bedrock present under each sampling pit. The numbers of soil sampling pits available for each class were nine for Blue Ridge siliciclastic, three for Blue Ridge granitic, three for Blue Ridge basaltic, and three for Valley and Ridge siliciclastic.

Atmospheric deposition data

Total atmospheric deposition consists of three components: wet deposition, the flux of ions occurring in precipitation; dry deposition, resulting from gaseous and particulate fluxes; and cloud/fog deposition, which can be particularly important in mountainous areas. Estimates of total deposition are needed for each site in the study. Wet deposition measurements are available only for two sites in the study area, both in the Shenandoah National Park (Fig. 1). We derived estimates of precipitation volume and of wet deposition of ions for each of the 60 sites from the deposition model of Grimm and Lynch (1997). Their model provides spatially continuous estimates of precipitation volume and of sulfate, nitrate, chloride, hydrogen ion, calcium, ammonium, magnesium, potassium, and sodium concentration in precipitation using interpolated data from a national network of precipitation monitoring stations (the National Acid Deposition Program monitoring sites) and weighted least-squares regression techniques to account for the influence of topography and elevation. At the two sites for which wet deposition data are available, the estimates derived from the Grimm and Lynch (1997) model agree well with the observed values for deposition of sulfate, nitrate, and ammonium ions (ratio of measured to estimated wet deposition = 1.0 for sulfate, 1.0 for nitrate, and 0.8 for ammonium ions).

There are no observations of dry deposition or cloud/fog deposition for any site included in this study. Estimates of dry deposition fluxes and cloud/fog deposition fluxes of sulfate and nitrate have, however, been published for single years in Shenandoah National Park (Vong et al. 1991; Clarke et al. 1997). We combined those estimates and the observed wet deposition in the park for the same years to calculate the ratio of estimated total deposition to the observed wet deposition for sulfate, nitrate, and ammonium ions. We used these ratios (called dry deposition factors) for all 60 streams in this study to calculate total deposition of sulfate, nitrate, and ammonium ions from the estimated wet deposition data. The dry deposition factors used were 2.0 for sulfate, 2.5 for nitrate, and 2.5 for ammonium and are consistent with published values used in previous studies in this region (e.g., Cosby et al. 1985*b*).

Calibration of the model (and estimation of the historical brook trout status of the streams) required a temporal sequence of historical anthropogenic deposition. Such long-term, continuous historical deposition data do not exist. We used historical emissions data as a surrogate for deposition. The emissions for each year in the historical period were normalized to emissions in 1991(a year for which observed deposition data are available). Using this scaled sequence of emissions, historical deposition was estimated by multiplying the total deposition estimated for each site in 1991 by the emissions scale factor for any year in the past to obtain deposition for that year. Emissions data for both SO_2 and NO_x are available on a state by state basis back to the turn of the century (see the U.S. Environmental Protection Agency web site). Yearly emissions from all states comprising the U.S. Environmental Protection Agency mid-Atlantic region were added together to produce a "regional" emissions history for the sites in western Virginia.

Three scenarios of future acidic deposition were considered in this study: constant deposition at 1991 levels and two levels of reduced acidic deposition, 40 and 70% reductions from 1991 levels. The scenarios are based only on changes in sulfate deposition; all other ions in deposition for each stream are assumed to remain constant into the future at 1991 levels. We chose the 40% reduction because that is approximately the national level of emissions reductions (and presumably deposition reductions) expected as a result of the 1990 U.S. Clean Air Act amendments. The 70% percent reduction scenario (relative to 1991) represents the net effect of reducing the residual deposition left after the 40% reduction by half. Possible changes in nitrogen deposition were not considered in this study. Although nitrogen deposition may contribute to surface water acidification in some areas, nitrogen is effectively immobilized by forest regeneration in most of western Virginia's upland watersheds. As a result, nitrate concentrations in Virginia brook trout streams are low (Webb et al. 1995), and in the absence of watershed disturbance, the potential for increasing concentrations of nitrate is thought to be minimal (Stoddard 1994). For each scenario of reduced sulfur deposition, simulations were run for 50 years into the future. For the two scenarios assuming reduced deposition, the sulfate deposition reductions were implemented linearly over the first 20 years, with constant sulfate deposition at the reduced level assumed for the final 30 years of simulation. Output from the future simulations are examined at two times, 20 and 50 years. The former represents the responses of the streams immediately at the completion of the deposition reduction and might be considered the "direct" effect of the assumed reduction. The latter is included to examine "delayed" effects that might occur as a result of continued deposition at the reduced levels.

Results

Current and past conditions

Based on the observed volume-weighted annual average ANC for 1991, a large number of trout streams in western Virginia currently have moderate to severe problems with acidity (Fig. 2). Only 50% of the streams are currently not acidic and thus have water quality that is suitable for brook trout. Approximately one third (30%) of all trout streams in Virginia are marginal or unsuitable for brook trout because they are currently either episodically (24%) or chronically acidic (6%). In addition to this 30%, another 20% of the streams (indeterminate streams, Fig. 2) may or may not be experiencing difficulties.

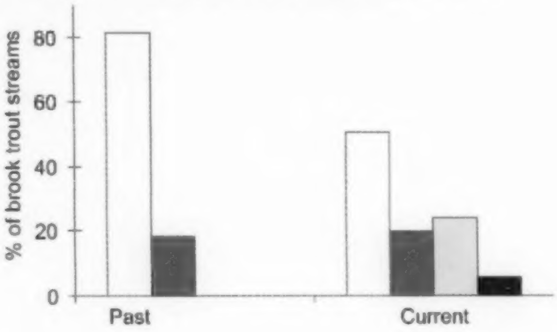
How much of this current condition is attributable to the effects of industrially generated acidic deposition can be estimated by examining the reconstructed water quality conditions of the streams. Based on the model simulations, 82% of these streams would not have been acidic prior to the onset of acidic deposition and would have been suitable for brook trout (Fig. 2). The 18% of streams for which the reconstructed water quality is indeterminate probably were suitable for trout because there would have been no source of strong acid during storms and thus no possibility of episodic acidification in these marginal streams. The historical simulations produced no streams with chronic or episodic acidification problems.

Responses of streams to future deposition reductions

Approximately 50% of the streams considered in this study are currently not acidic and are suitable for brook trout. All three of the future deposition scenarios produced small declines in the number of suitable trout streams in the short term (20 years) (Fig. 3A). The responses at 50 years are decreases from 50% currently to 49% following a 70% reduction, to 45% following a 40% reduction, and to 42% assuming constant deposition at 1991 levels. This delayed response is due to leaching of accumulated sulfate in soils, with concomitant loss of base cation buffering.

Approximately 30% of all trout streams in western Virginia are either currently chronically (6%) or episodically (24%) acidic and thus are either marginal or unsuitable for brook trout. Streams in these categories have lower species richness, and trout in these streams will be affected by sublethal and (or) lethal stress. For 0 and 40% reductions, slight increases in the number of streams in these categories are forecast for the first 20 years, while the 70% reduction scenario results in no increase in the number of streams in these categories in the short term (Fig. 3B). The forecast long-term responses (50 years), however, show larger changes for these combined categories. For no deposition reduction, an additional 17% (from 30 to 47%) of streams will become marginal or unsuitable for brook trout. This translates approximately to an additional 50 streams. Even the 40% reduction scenario still results in an increase of 10% of streams (approximately 30 streams) that are marginal or unsuitable for brook trout.

Fig. 2. Current and past status of brook trout streams in western Virginia. Four categories of brook trout stream status are considered based on annual average ANC classes in the streams (see Table 1): suitable (open bars), indeterminate (lined bars), marginal (gray bar), and unsuitable (black bar). Current conditions are based on annual average ANC values calculated from 5 years (1989–1993) of quarterly stream sampling in the region. Past conditions are derived from model reconstructions of stream water ANC for streams in the region. Percentages are based on the population of all brook trout streams in Virginia that do not lie primarily on limestone and (or) have not been significantly affected by human activity within the watersheds (n = 304).



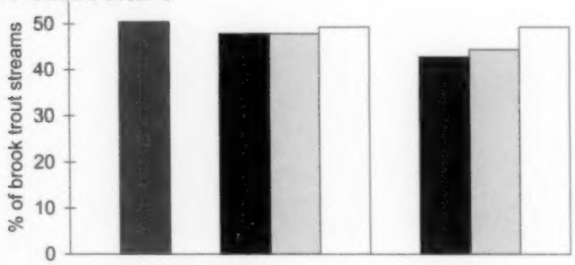
The effects of the deposition scenarios were greatest on the number of streams that are currently unsuitable for brook trout. There are large short-term as well as long-term changes in the percentage of these chronically acidic streams (Fig. 3C). For instance, the 40% reduction scenario results in more than a doubling of the number of chronically acidic streams in the first 20 years (from 6 to 13%) and nearly a quadrupling of such streams (to 22%) by the end of 50 years. The model projections also suggest that even a 70% reduction in deposition is not enough to prevent increases in the number streams unsuitable for brook trout. The number of chronically acidic streams is forecast to increase under the 70% reduction scenario from 6 to 11%, and that doubling is relatively rapid, occurring by the 20th year.

Discussion

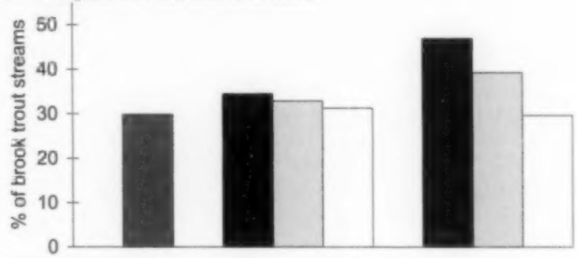
Our analysis of a regionally representative sample of brook trout streams suggests that only approximately half of all trout streams in western Virginia (on noncarbonate bed-

Fig. 3. Projected future status of brook trout streams in western Virginia. Three categories of future brook trout stream status are considered based on annual average ANC classes in the streams (see Table 1): (A) suitable, (B) combined marginal and unsuitable, and (C) unsuitable only. The projections are based on simulated stream water ANC values in response to three future sulfate deposition scenarios (the timing is described in the text): no change (black bars), a 40% reduction from current values (gray bars), and a 70% reduction from current values (open bars). Projections are presented for the 20th year and the 50th year of each scenario. Current observed brook trout stream status is presented as a reference (lined bars). Percentages are based on the population of all brook trout streams in Virginia that do not lie primarily on limestone and (or) have not been significantly affected by human activity within the watersheds (n = 304).

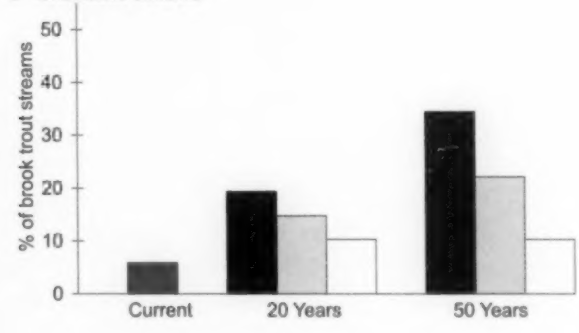
A Suitable streams



B Marginal and unsuitable streams



C Unsuitable streams



rock and with minimal land-use disturbance) can currently be safely assumed to be suitable for brook trout, with respect to the deleterious effects of acidic deposition. Our comparison of observed stream water quality with reconstructed water quality suggests that acidic deposition is responsible for ecological damage in approximately one third of brook trout streams in western Virginia. This percentage represents more than 100 streams.

Our model projections suggest that neither the 40 nor the 70% reductions in acidic deposition will increase the number of streams that are suitable for brook trout above the current 50%. In fact, the results in Fig. 3A strongly suggest that a 70% reduction in deposition is needed in the long term just to maintain the current number of streams that are not acidic and suitable for brook trout. Recovery of any of the 32% of suitable streams that have been lost due to acidification (82% historical reconstruction versus 50% current observations) is not likely unless deposition reductions in excess of 70% are achieved.

Our model projections also suggest that neither the 40 nor the 70% reduction scenarios will decrease the number of streams that are currently marginal or unsuitable for brook trout (i.e., there will be little recovery of lost stream resources at these reduction levels). In fact, the results in Fig. 3B strongly suggest that a 70% reduction in deposition is needed simply to prevent further increases in the number of marginal and unsuitable streams. Recovery of streams that are currently episodically or chronically acidic is not likely unless deposition reductions of greater than 70% are achieved.

As streams move from episodically to chronically acidic, the biological effects are more severe (e.g., loss of more fish and invertebrate species) and the streams become unsuitable for brook trout (Bulger et al. 1999). Practical remedial actions such as liming become more expensive and complicated as stream acidity becomes chronic, making management of these streams for fish resources problematic. Because the chronically acid streams are incapable of sustaining populations of brook trout and many other aquatic animals, it is worthwhile to express their numbers in absolute terms. For the population of brook trout streams to which this analysis applies (more than 300 streams), our projections suggest that no reduction in acidic deposition from 1991 levels will result in an additional 70 streams becoming chronically acidic and unsuitable for brook trout (6% currently versus 35% after 50 years). Projections with a 40% reduction result in an additional 48 streams becoming chronically acidic (6% currently versus 22% after 50 years). Even assuming a 70% reduction, an additional 15 streams will likely become chronically acidic (6% currently versus 11% after 50 years). Due to the length of time required to restore buffering capacity in watershed soils, most of these streams will remain acidified for the foreseeable future. Chronically acidic streams may be truly "lost" fisheries resources, since brook trout is the most acid-tolerant, stream-dwelling fish species in the region (Jenkins and Burkhead 1993).

Our analysis deals with probable negative effects of acidification specifically on brook trout, not just at the time of the assessment but also projected into the past and future. Both brown trout and rainbow trout as well as other ecologically important species, including many minnows, darters, and aquatic insects, are less acid-tolerant than brook trout (Heard et al. 1997; Bulger et al. 1999), so negative effects of acidification on these species are anticipated under conditions still tolerable for brook trout. Our projections indicate that at least a 70% reduction in total sulfate deposition (relative to

1991 levels) is necessary to prevent further damage to brook trout streams in Virginia. Even this reduction will not increase the number of streams suitable for brook trout and in fact will not prevent some already partially damaged streams from becoming chronically acidic, or essentially "fishless." The SAA (SAMAB 1996) concluded that 59% of about 53 000 km of potential wild trout streams are in areas highly vulnerable to acidification. If our results for Virginia are extrapolated to the entire 15.4×10^6 ha area of the Southern Appalachians, current and potential future losses to acidification are substantial and can be measured in thousands of kilometres of trout stream.

Acknowledgements

This work was partially funded by Trout Unlimited; we gratefully acknowledge their support and helpful discussions. We thank the other investigators involved in the Shenandoah Watershed Study and the Fish in Sensitive Habitat Study (at the University of Virginia and Virginia Polytechnic Institute and State University) for the use of their data and for many helpful comments. Partial funding for this work was also provided by the U.S. National Park Service.

References

Altshuller, A.P., and Linthurst, R.A. 1984. The acidic deposition phenomenon and its effects: critical assessment review papers. EPA/600/8-83/016BF. U.S. Environmental Protection Agency, Washington, D.C.

Bulger, A.J., Dolloff, C.A., Cosby, B.J., Eshleman, K.N., Webb, J.R., and Galloway, J.N. 1995. The "Shenandoah National Park: Fish in Sensitive Habitats (SNP: FISH)" Project. An integrated assessment of fish community responses to stream acidification. Water Air Soil Pollut. **85**: 309–314.

Bulger, A.J., Cosby, B.J., and Webb, R. 1998. Acid rain: current and projected status of coldwater fish communities in the southeastern U.S. in the context of continued acid deposition. A Coldwater Conservation Fund Report. Trout Unlimited, Arlington, Va.

Bulger, A.J., Cosby, B.J., Dolloff, C.A., Eshleman, K.N., Webb, J.R., and Galloway, J.N. 1999. The "Shenandoah National Park: Fish in Sensitive Habitats (SNP: FISH)" Project. Final Report. Vols. I–IV plus interactive computer model. (Available on CD-Rom from John F. Karish, National Park Service, Northeast Field Area, 209B Ferguson Building, University Park, PA 16802-4301, U.S.A.)

Church, M.R., Shaffer, P.W., Thornton, D.L., Cassell, D.L., Liff, C.I., Johnson, M.G., Lammers, D.A., Lee, J.J., Holdren, G.R., Kern, J.S., Liegel, L.H., Pierson, S.M., Stevens, D.L., and Rochelle, B.P. 1992. Direct/Delayed Response Project: future effects of long-term sulfur deposition on surface water chemistry in the Mid-Appalachian region of the eastern United States. EPA/600/R-92/186. U.S. Environmental Protection Agency, Washington, D.C.

Clarke, J.F., Edgerton, E.S., and Martin, B.F. 1997. Dry deposition calculations for the Clean Air Status and Trends Network. Atmos. Environ. **31**: 3667–3678.

Cosby, B.J., Wright, R.F., Hornberger, G.M., and Galloway, J.N. 1985*a*. Modelling the effects of acid deposition: assessment of a lumped parameter model of soil water and streamwater chemistry. Water Resour. Res. **21**: 51–63.

Cosby, B.J., Wright, R.F., Hornberger, G.M., and Galloway, J.N. 1985*b*. Modelling the effects of acid deposition: estimation of long-term water quality responses in a small forested catchment. Water Resour. Res. **21**: 1591–1601.

Cosby, B.J., Hornberger, G.M., Galloway, J.N., and Wright, R.F. 1985*c*. Time scales of catchment acidification: a quantitative model for estimating freshwater acidification. Environ. Sci. Technol. **19**: 1144–1149.

Cosby, B.J., Wright, R.F., and Gjessing, E. 1995. An acidification model (MAGIC) with organic acids evaluated using whole-catchment manipulations in Norway. J. Hydrol. **170**: 101–122.

Cosby, B.J., Norton, S.A., and Kahl, J.S. 1996. Using a paired-watershed manipulation experiment to evaluate a catchment-scale biogeochemical model. Sci. Total Environ. **183**: 49–66.

Dennis, T.E., and Bulger, A.J. 1995. Condition factor and whole-body sodium concentrations in a freshwater fish: evidence for acidification stress and possible ionoregulatory over-compensation. Water Air Soil Pollut. **85**: 377–382.

Dennis, T.E., MacAvoy, S.E., Steg, M.B., and Bulger, A.J. 1995. The association of water chemistry variables and fish condition in streams of Shenandoah National Park (U.S.A.). Water Air Soil Pollut. **85**: 365–370.

Freda, J., Sanchez, D.A., and Bergman, H.L. 1991. Shortening of branchial tight junctions in acid-exposed rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **48**: 2028–2033.

Gagen, C.J., and Sharpe, W.E. 1987. Net sodium loss and mortality of three salmonid species exposed to a stream acidified by atmospheric deposition. Bull. Environ. Contam. Toxicol. **39**: 7–14.

Gibson, J.H., Galloway, J.N., Schofield, C., McFee, W., Johnson, S., McCarely, S., Dise, N., and Herzog, D. 1983. Rocky Mountain acidification study. U.S. Fish and Wildlife Service Reprint no. FWS/OBS-8-/40.17. U.S. Department of the Interior, Fish and Wildlife Service, Eastern Energy and Land Use Team, Route 3, Box 44, Kearneysville, WV 25430, U.S.A.

Grimm, J.W., and Lynch, J.A. 1997. Enhanced wet deposition estimates using modeled precipitation inputs. Final Report to the USDA Forest Service, Northeast Forest Experiment Station, Northern Global Change Research Program (23-721). (Available from J.W. Grimm, The Pennsylvania State University, School of Forest Resources, 311 Forest Resources Lab, University Park, PA 16802, U.S.A.)

Heard, R.M., Sharpe, W.E., Carline, R.F., and Kimmel, W.G. 1997. Episodic acidification and changes in fish diversity in Pennsylvania headwater streams. Trans. Am. Fish. Soc. **126**: 977–984.

Herlihy, A.T., Kaufmann, P.R., Church, M.R., Wigington, P.G., Jr., Webb, J.R., and Sale, M.J. 1993. The effects of acidic deposition on streams in the Appalachian Mountain and Piedmont region of the mid-Atlantic United States. Water Resour. Res. **29**: 2687–2703.

Herlihy, A.T., Kaufmann, P.R., Stoddard, J.L., Eshleman, K.N., and Bulger, A.J. 1996. Effects of acidic deposition on aquatic resources in the Southern Appalachians with a special focus on Class I wilderness areas. The Southern Appalachian Mountain Initiative (SAMI), 57 Woodfin Place, Asheville, NC 28801, U.S.A.

Hyer, K.E., Webb, J.R., and Eshleman, K.N. 1995. Episodic acidification of three streams in Shenandoah National Park, Virginia, U.S.A. Water Air Soil Pollut. **85**: 523–528.

Jenkins, A., Whitehead, P.G., Musgrove, T.J., and Cosby, B.J. 1990. A regional model of acidification in Wales. J. Hydrol. **116**: 403–416.

Jenkins, R.E., and Burkhead, N.M. 1993. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Md.

Kaufmann, P.R., Herlihy, A.T., Elwood, J.W., Mitch, M.E., Overton, W.S., Sale, J.J., Messer, J.J., Cougan, K.A., Peck, D.V., Reckhow, K.H., Kinney, A.J., Christie, S.J., Brown, D.D., Hagley, C.A., and Jager, H.I. 1988. Chemical characteristics of streams in the mid-Atlantic and southeastern United States. Vol. I. Population descriptions and physico-chemical relationships. Rep. 600/3-88/021a. U.S. Environmental Protection Agency, Washington, D.C.

Knapp, C.M., Heimbuch, D.G., Greening, H.S., and Filbin, G.J. 1988. Maryland synoptic stream survey. AD-88-2. Prepared by International Science and Technology, Inc., for the Maryland Department of Natural Resource, Power Plant Research Program, Annapolis, Md.

MacAvoy, S.E., and Bulger, A.J. 1995. Survival of brook trout (*Salvelinus fontinalis*) embryos and fry in streams of different acid sensitivity in Shenandoah National Park, U.S.A. Water Air Soil Pollut. **85**: 445–450.

Mohn, L.O., Kauffman, J.W., and Bugas, P.E. 1989. Effects of acidification on bottom fauna and fish populations in St. Mary's River, Augusta County, VA. *In* Abstracts, Annual Meeting of the American Fisheries Society, 1989. American Fisheries Society, Bethesda, Md.

National Acid Precipitation Assessment Program (NAPAP). 1990. Integrated assessment report. National Acid Precipitation Assessment Program, Washington, D.C.

National Acid Precipitation Assessment Program (NAPAP). 1991. The U.S. National Acid Precipitation Assessment Program 1990 integrated report. NAPAP Office of the Director, 722 Jackson Place NW, Washington, DC 20503, U.S.A.

Norton, S.A., Wright, R.F., Kahl, J.S., and Schofield, J.P. 1992. The MAGIC simulation of surface water at, and first year results from, the Bear Brook watershed manipulation, Maine, U.S.A. Environ. Pollut. **77**: 279–286.

Schindler, D.W. 1988. Effects of acid rain on freshwater ecosystems. Science (Washington, D.C.), **239**: 149–157.

Southern Appalachian Man and the Biosphere (SAMAB). 1996. Southern Appalachian Assessment Aquatics Technical Report 2 of 5. U.S. Department of Agriculture, Forest Service, Southern Region, Atlanta, Ga.

Stoddard, J.L. 1994. Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. *In* Environmental chemistry of lakes and reservoirs. *Edited by* L.A. Baker. Advances in Chemistry Series No. 237. American Chemical Society, Washington, D.C.

Sullivan, T.J., Bernert, J.A., Jenne, E.A., Eilers, J.M., Cosby, B.J., Charles, D.F., and Selle, A.R. 1991. Comparison of MAGIC and diatom paleolimnological model hindcasts of lakewater acidification in the Adirondack region of New York. Prepared for the U.S. Department of Energy under contract DE-AC06-76RLO 1830. Pacific Northwest Laboratory, Richland, Wa.

Sullivan, T.J., Cosby, B.J., Driscoll, C.T., Charles, D.F., and Hemond, H.F. 1996. Influence of organic acids on model projections of lake acidification. Water Air Soil Pollut. **91**: 271–282.

Thornton, K., Marmorek, D., and Ryan, P. 1990. Methods for projecting future changes in surface water acid–base chemistry. *In* Acidic deposition: state of science and technology. Rep. 14. *Edited by* P.M. Irving. National Acid Precipitation Assessment Program, Washington, D.C.

Vong, R.J., Sigmon, J.T., and Mueller, S.F. 1991. ES&T critical review: cloud water deposition to Appalachian forests. Environ. Sci. Technol. **25**: 1014–1021.

Walsh, S.J., Burkhead, N.M., and Williams, J.D. 1995. *In* Our living resources. *Edited by* E.T. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac. U.S. Department of the Interior, National Biological Service, Washington, D.C. pp. 144–147.

Webb, J.R., Deviney, F.A., Galloway, J.N., Rinehart, C.A., Thompson, P.A., and Wilson, S. 1994. The acid–base status of native brook trout streams in the mountains of Virginia: a regional assessment based on the Virginia Trout Stream Sensitivity Study. Report to the Virginia Department of Game and Inland Fisheries, P.O. Box 11104, Richmond, VA 23230, U.S.A.

Webb, J.R., Cosby, B.J., Deviney, F.A., Eshleman, K.N., and Galloway, J.N. 1995. Change in the acid–base status of an Appalachian Mountain catchment following forest defoliation by the gypsy moth. Water Air Soil Pollut. **85**: 535–540.

Winger, P., Lasier, P.J., Hurdy, M., Fowler, D., and Van Den Avyle, J.J. 1987. Sensitivity of high-elevation streams in the Southern Blue Ridge Province to acidic deposition. Water Resour. Bull. **23**: 379–386.

Wright, R.F., Cosby, B.J., Ferrier, R.C., Jenkins, A., Bulger, A.J., and Harriman, R. 1994. Changes in acidification of lochs in Galloway, southwestern Scotland 1979–88: the MAGIC model used to evaluate the role of afforestation, calculate critical loads and precite fish status. J. Hydrol. **161**: 275–285.

Food web structure and nutrient enrichment: effects on sediment phosphorus retention in whole-lake experiments

Jeffrey N. Houser, Stephen R. Carpenter, and Jon J. Cole

Abstract: A series of whole-lake manipulations of both food web structure and nutrient loading rate revealed that the downward vertical P flux was significantly affected by food web structure. Food webs and nutrient input rates of two lakes were manipulated while a third lake served as a reference system. Phosphorus transport to the sediments was examined by three independent methods: mass-balance budgets, sediment traps, and sediment cores. After 2 years of pretreatment study, manipulated lakes were fertilized for 5 years at rates that were similar within each year but varied among years from 0.97 to 6.0 mg $P \cdot m^{-2} \cdot day^{-1}$. Increased vertical P flux was associated with the increased abundance of large-bodied zooplankton grazers such as *Daphnia pulex*. Vertical P flux increased with P input rates, but the increase was often insufficient to prevent P accumulation in the water column. Sediment trap measurements of the vertical P flux were significantly higher than the long-term sediment P retention measured by sediment cores and the seasonal sediment P retention calculated by mass balance. Using ^{210}Pb data from the sediment cores, we corrected the sediment trap measurements of vertical P flux for focusing and brought them into better agreement with the seasonal sediment P retention calculated by mass balance.

Résumé : Une série de manipulations panlacustres de la structure du réseau trophique et du taux de charge des nutriants a révélé que le flux vertical descendant du phosphore (P) était affecté de façon significative par la structure du réseau trophique. Les réseaux trophiques et les taux d'apport de nutriants ont été manipulés dans deux lacs, tandis qu'un troisième lac servait de système témoin. Nous avons examiné le transport de P vers les sédiments par trois méthodes indépendantes : bilans massiques, pièges à sédiments, et carottes de sédiments. Après 2 ans d'étude prétraitement, les lacs ont été fertilisés pendant 5 ans à des taux qui étaient semblables au cours d'une année, mais qui variaient d'une année à l'autre de 0,97 à 6,0 mg $P \cdot m^{-2} \cdot jour^{-1}$. L'augmentation du flux vertical de P était associée à un accroissement de l'abondance des brouteurs zooplanctoniques de grande taille comme *Daphnia pulex*. Le flux vertical de P augmentait avec l'apport de P, mais cette augmentation était souvent insuffisante pour empêcher l'accumulation de P dans la colonne d'eau. Les mesures du flux vertical de P dans les pièges à sédiments étaient nettement supérieures à la rétention à long terme de P dans les sédiments mesurée par les carottes de sédiments, et à la rétention saisonnière de P dans les sédiments calculée par le bilan massique. À l'aide de données sur ^{210}Pb obtenues dans les carottes de sédiments, nous avons corrigé les mesures du flux vertical de P obtenues avec les pièges à sédiments pour tenir compte de la focalisation, ce qui a rapproché ces valeurs de la rétention saisonnière de P dans les sédiments calculée à l'aide du bilan massique.

[Traduit par la Rédaction]

Introduction

Food web structure can affect nutrient fluxes in lakes by multiple mechanisms. Differences in fish communities affect the amount of P stored in fish biomass and the amount of P recycled into the water column (Schindler et al. 1993). Zooplankton can affect the downward vertical flux (hereafter referred to as vertical flux) of nutrients in the water column by grazing and subsequent egestion (e.g., Paffenhöfer and Knowles 1979; Uehlinger and Bloesch 1987; Elser et al. 1995), by changing the phytoplankton size distributions (and therefore sinking velocities) due to selective grazing (Bloesch and Bürgi 1989), and by the sinking of dead zooplankton (Reinertsen et al. 1990).

Previous studies of the effects of food webs on sedimentation, using experimental enclosures, whole-lake observations, and theoretical models, have shown that zooplankton can have either positive or negative effects on sedimentation (e.g., Bürgi et al. 1979; Bloesch and Bürgi 1989; Mazumder et al. 1989). In previous whole-lake studies, abundant zooplankton increased the settling velocity of particles (sedimentation rate divided by euphotic zone concentration), but because large grazers reduced the concentration of particulate P (PP), vertical P flux declined (Reinertsen et al. 1990; Elser et al. 1995; Sarnelle 1999). Thus, the impact of zooplankton on vertical flux may depend on the relative magnitude of the effects on P settling velocity and PP concentration. Current theory also suggests that the direction

Received June 8, 1999. Accepted March 23, 2000.
J15176

J.N. Houser[1] and S.R. Carpenter. Center for Limnology, University of Wisconsin, 680 N. Park St., Madison, WI 53706, U.S.A.
J.J. Cole. Institute of Ecosystem Studies, Box AB, Route 44A, Milbrook, NY 12545, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: jnhouser@students.wisc.edu

of the effect of zooplankton on sedimentation depends on the sedimentation rates of zooplankton fecal material relative to phytoplankton (Elser et al. 1995; Sarnelle 1999). Our whole-lake experiments, conducted over 6 years, provided an opportunity to examine the effects of the food web on vertical P flux over time scales that are frequently used for ecosystem analyses and lake management (i.e., annual budgets).

Mass-balance P budgets are a classic approach to studying nutrient fluxes in lakes (e.g., Schindler et al. 1987). The rate of P loss due to permanent retention in the sediments is a particularly difficult component to measure (Sas 1989; Dillon et al. 1990). For the purpose of predicting the effect of changes in P input rate on the lake P concentration, P loading models have been used to circumvent the difficulties of measuring sediment P retention. These models estimate sediment P retention as a constant proportion of either the P mass in the lake using a P sedimentation coefficient (σ) (Vollenweider 1976; Canfield and Bachmann 1981) or the P input to the lake using a P retention coefficient (R_P) (Dillon and Rigler 1974; Prairie 1988). Sedimentation and retention coefficients assume that the proportion of water column P mass or P input permanently retained by the sediments is constant over a range of P input rates and is independent of food web structure.

Various approaches have been used to estimate whole-lake sediment P retention more directly. Sediment traps can measure vertical flux of particles on short time scales (days to weeks) (e.g., Bloesch and Burns 1980). However, the relationship between the vertical flux measured by sediment traps and the whole-lake sediment P retention is usually unknown and may be obscured by sediment resuspension and focusing (Davis 1968; Dillon et al. 1990; Dymond et al. 1996). Sediment focusing occurs when sediments in the shallower regions of a lake are resuspended and settle in the deeper regions of the lake, producing higher rates of sediment accumulation in the deep, central part of a lake basin than in the shallow perimeter (Likens and Davis 1975). Considerable effort has been devoted towards quantifying and predicting sediment focusing (e.g., Hilton 1985; Bloesch 1994), yet the use of sediment traps for measuring sediment P retention remains questionable. As a result, other approaches, including sediment cores (e.g., Evans and Rigler 1980; Dillon and Evans 1993; Rowan et al. 1995) and mass-balance budgets (Cross and Rigler 1983; Schindler et al. 1987; Dillon and Evans 1993), have been used to determine sediment P retention.

Several studies have compared some of these different methods of measuring whole-lake sediment P retention. Evans and Rigler (1980) and Dillon and Evans (1993) found reasonable agreement between estimates derived from sediment cores and from mass-balance budgets. In contrast, vertical P flux measured by sediment traps is frequently much higher than sediment P retention determined from sediment cores (Davis 1968; Bloesch and Evans 1982) and from mass-balance budgets (Dillon et al. 1990).

We examined effects of changes in P input rate and food web structure on P vertical flux (using sediment traps), P retention in the sediments (using mass-balance budgets and sediment cores), and P accumulation in the water column in experimentally manipulated lakes. We hypothesized that (*i*) piscivore-dominated food webs with abundant efficient grazers (e.g., *Daphnia pulex*) would be associated with reduced vertical P flux and (*ii*) vertical P flux and sediment P retention would increase in proportion to increases in P input rate.

Methods

Study site

Paul, Peter, and West Long lakes are small, steep-sided basins located at the University of Notre Dame Environmental Research Center (UNDERC) (46°13′N, 89°32′W) near Land o' Lakes, Wisconsin, U.S.A. (Table 1). These lakes have been studied since 1951 and are well described in the literature (Carpenter and Kitchell 1993; Carpenter et al. 1996).

Whole-lake manipulations

The manipulations have been described in detail elsewhere (Carpenter et al. 1996) and are described briefly here. Paul and Peter lakes were separated by an earthen dike in 1951. In 1991, Long Lake was divided into two basins (East and West Long lakes) separated by a shallow channel (Central Long Lake) using two plastic curtains. One of the curtains was removed in September 1995, and the shallow channel became part of West Long Lake. The curtain removal had no obvious effects on East or West Long Lake.

The food webs of Peter and West Long lakes have been manipulated into contrasting configurations (Table 1) (for details, see Carpenter et al. 1996). The fish community of Peter Lake is dominated by planktivorous fishes (e.g., golden shiner (*Notemigonus crysoleucas*) and fathead minnow (*Pimephales promelas*)) that exert intense size-selective predation pressure on the zooplankton community. Consequently, the zooplankton community in Peter Lake is dominated by small-bodied zooplankton such as *Bosmina* spp., *Daphnia rosea*, and *Diacyclops thomasi*. The fish in West Long Lake are predominantly piscivorous bass (largemouth bass (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*)) resulting in abundant large-bodied zooplankton, e.g., *D. pulex*. Paul Lake (the reference lake) is dominated by largemouth bass and large-bodied zooplankton such as *D. pulex* and *Holopedium gibberum*.

Beginning in 1993, Peter and West Long lakes were enriched with N and P (N:P by atoms exceeded 30). Because one of the goals of the experiment was to determine the threshold for grazer control of chlorophyll concentrations (Carpenter et al. 1996), the rate of P enrichment was varied among years from approximately 1 to 6 $mg \cdot m^{-2} \cdot day^{-1}$, or approximately 3–20 times the pretreatment P input rate (Fig. 1).

Phosphorus budgets

Phosphorus budgets were constructed for the summer field season (approximately 15 May – 15 September) for Paul, Peter, and West Long lakes according to a mass-balance model:

$$\Delta M_P = I_P - O_P - S_P$$

where ΔM_P is the change in P mass in the lake during the field season (kilograms), the P input (I_P) includes the natural P input to the lake from the watershed plus the experimental P enrichment (kilograms), O_P is the P outflow from the lake (kilograms), and S_P is the sediment P retention (kilograms).

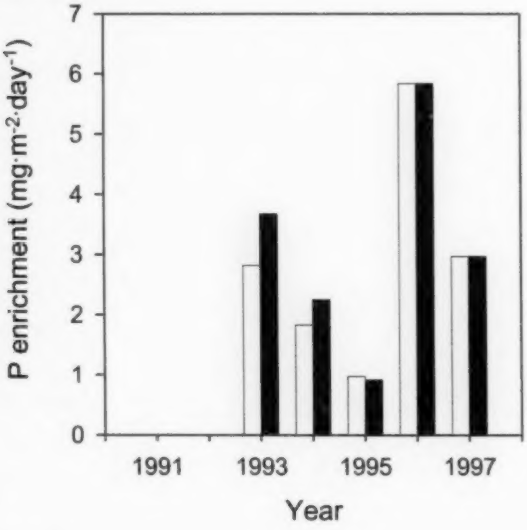
To determine the mass of P in the water column, weekly water samples were taken from seven depths and analyzed for total P (TP) using a persulfate digestion followed by analysis (ascorbic acid method) using a Lachat AE autoanalyzer. The P mass in the water column was calculated for each sampling date using the vertically integrated TP concentration and lake hypsometric curves. The change in water column P mass for the stratified season was the difference between the P mass on the first and last sampling

Table 1. Limnological characteristics of the study lakes.

Characteristic	Paul Lake	Peter Lake	West Long Lake
Area (ha)	1.7	2.7	3.4
Mean depth (m)	3.7	5.7	4.7
Maximum depth (m)	12.8	19.9	17.0
Mean thermocline depth (m) (1991–1997)	3.6	3.5	3.2
Proportion of lake volume in hypolimnion (1991–1997)	0.36	0.54	0.48
Mean water residence time (days) (SD)[a]	—	835 (372)	849 (441)
Preenrichment (1991–1992) epilimnetic chlorophyll ($\mu g \cdot L^{-1}$)	4.33	3.93	4.59
Enrichment (1993–1997) epilimnetic chlorophyll ($\mu g \cdot L^{-1}$)	3.87	28.9	12.4
Dominant fish	Piscivorous bass	Planktivorous minnows	Piscivorous bass
Dominant zooplankton	Large grazers (e.g., *D. pulex*, *H. gibberum*)	Small grazers (e.g., *Bosmina* spp.)	Large grazers (e.g., *Daphnia* spp.)

[a] Residence time shown is the mean for 1992–1996 for West Long Lake and 1993–1996 for Peter Lake. Residence time was not measured in Paul Lake.

Fig. 1. Rates of P enrichment for Peter (open bars) and West Long (solid bars) lakes. Paul Lake, the reference lake, received no enrichment and is not shown. The lakes were not enriched in 1991 and 1992.



dates of the season (approximately mid-May and early September, respectively).

The natural component of I_P was estimated by two different methods: Vollenweider's (1976) loading equation and mass balance. Vollenweider's (1976) equation is

$$L_P = [P]q_s(1 + (q_s/z)^{0.5})$$

where L_P, the quantity to be estimated, is the natural P input to the lake (milligrams per square metre per year), [P] is the lake P concentration (milligrams per cubic metre), q_s is the hydraulic load (metres per year) (determined by Cole and Pace (1998) using LiBr, a hydraulic tracer), and z is the mean depth (metres). For the enriched lakes (Peter and West Long lakes), the natural P input was estimated in 1991 and 1992 (preenrichment years) and the mean of 1991 and 1992 used as the estimate of natural P input in subsequent years. For the reference lake (Paul Lake), the natural P input rate was estimated for all years. In the enrichment years, the natural input rate is low relative to the experimental P additions and is a very small component of the lake P budgets. We also estimated P input using the above mass-balance equation (rearranged to $I_P = \Delta M_P + O_P + S_P$), direct measures of P flux to the sediments (sediment traps, sediment cores), and the P budget data (which included the change in P mass and the P outflow).

The P outflow was estimated using the integrated epilimnetic TP concentration and the water outflow from the lakes. The outflow was determined by Cole and Pace (1998) using LiBr. LiBr was not added to Paul Lake because it serves as the reference lake. Its outflow was approximated by assuming that it received the same water load (centimetres per day) as Peter Lake. The P outflow is a small component of the P budgets, and errors due to this approximation are negligible in the overall P budget.

Three different aspects of P flux to the sediments were measured. Sediment traps measured the short-term vertical P flux, and sediment cores were used to measure long-term sediment P retention. Mass-balance budgets were used to calculate seasonal sediment P retention, which was compared with vertical P flux measured by sediment traps and long-term sediment P retention measured by sediment cores. Each of these methods is described in detail below.

Mass-balance estimates of sediment P retention

Seasonal sediment P retention was calculated by rearranging the above mass-balance equation to

$$S_P = I_P - O_P - \Delta M_P.$$

Sediment traps

The vertical P flux was measured by analyzing samples from duplicate sediment traps, each consisting of three collection tubes (5 cm in diameter, 25 cm high). The sediment traps were suspended at 9 m (Paul Lake) or 12 m (Peter and West Long lakes), well below the thermocline, in the central part of lake and sampled biweekly. Sediment trap samples were analyzed for TP and corrected for hypolimnetic TP at depth. The sediment trap data were adjusted for sediment focusing as described below.

Table 2. Sedimentation rates and sediment characteristics calculated for individual cores and whole lakes.

Lake	Core depth (m)	Sediment accumulation rate (g·m^{-2}·year^{-1})	Surface sediment P concentration (mg·g^{-1})	^{210}Pb inventory (dpm·cm^{-2})	Lake ^{210}Pb inventory (dpm·cm^{-2})	^{210}Pb focusing
Peter	19.2	113.5	3.51	70.7	23.0	0.33
	14.0	40.5	1.87	51.0		
	7.0	40.4	1.92	30.0		
West Long	17.6	181.8	3.32	95.9	37.5	0.39
	11.3	108.7	3.65	67.8		
	6.6	147.5	2.32	48.8		

Note: Focusing is determined from the individual core ^{210}Pb inventory relative to the whole-lake inventory.

Sediment cores

Long-term sediment P retention was measured using sediment cores. Sediment cores were taken in January 1997 using a freeze-corer (Swain 1973). Three cores were taken from each of Paul, Peter, and West Long lakes. In each lake, the cores were distributed so that one was taken from the deep, central part of the lake, one from an intermediate depth, and one from a shallow depth (Table 2). Each frozen core was sliced in half lengthwise so that half of the core could be used for ^{210}Pb analysis and half for TP analysis. The cores were cleaned by removing the outer 1–2 mm with a wood plane and sliced into horizontal sections between 0.5 and 2.5 cm thick.

The ^{210}Pb analysis was performed by MyCore Labs, Ontario, Canada. Details are presented in Evans and Rigler (1980) with modifications described in Rowan et al. (1995). The concentrations of ^{210}Pb were interpreted using the constant rate of supply (CRS) ^{210}Pb dating model (Robbins 1978; Appleby et al. 1979). The CRS model assumes that the input of excess ^{210}Pb to the sediment–water interface has remained constant through time but allows for fluctuations in the deposition of sediment over time.

The two deeper cores in Peter and Paul lakes had clay layers from the 1951 construction of the dike and the subsequent partial washout of the dike in 1956. In Peter Lake, the sediment accumulation rates of the two deeper cores were estimated using both ^{210}Pb and the clay layer. Because the two methods produced similar estimates of sediment accumulation rates (Houser 1998), only the shallowest core in Paul Lake was dated using ^{210}Pb; the sediment accumulation rates of the two deeper cores in Paul Lake were determined based on the sediment that had accumulated above the 1951 clay layer.

For the TP analysis, cores were lyophilized and then sliced into horizontal sections. The P analysis was performed by the University of Wisconsin-Madison Soil and Plant Analysis Laboratory using a perchloric acid digestion and ICP spectrophotometry.

Whole-lake sediment accumulation

The ^{210}Pb inventory was calculated for the ^{210}Pb-dated cores from Peter and West Long lakes after Crusius and Anderson (1995), and preenrichment sediment P retention was calculated from the cores from all three experimental lakes. The preenrichment sediment P retention for each core was calculated using the average preenrichment sediment accumulation rate and the asymptotic P concentration that occurred several centimetres below the surface of the core. The concentration at this point several centimetres below the surface was considered to be representative of long-term P storage in the sediments.

Estimates of whole-lake ^{210}Pb inventories and sediment P retention were obtained in the following manner. The bottom of each lake was divided into isobaths at 1-m intervals. The area between each pair of isobaths was obtained from the morphometric maps of the lake. Linear interpolation by depth between cores was used to estimate a sediment accumulation rate for each 1-m depth increment. The sediment accumulation rate at 0 m was assumed to be 0 g·m^{-2}·year^{-1}. The sum of the rates determined for the area of each 1-m section was divided by the area of the whole lake to determine the average whole-lake value. Sediment focusing was estimated by comparing the ^{210}Pb inventory of the deepest central cores (disintegrations per minute per square centimetre (dpm·cm^{-2})) with the estimated whole-lake inventory (dpm·cm^{-2}).

Zooplankton

Zooplankton were sampled weekly by duplicate vertical hauls with a calibrated 80-μm-mesh Nitex net (30 cm in diameter). The samples were preserved with cold sugared formalin or Lugol's solution for later identification and enumeration, as described in detail in Carpenter and Kitchell (1993). The mean lengths of zooplankton were determined by measuring randomly selected individuals of each species until the standard error was less than 10% of the mean length for each species. Mean crustacean body length was used as an index of food web configuration and zooplankton grazing intensity (Pace 1984; Carpenter and Kitchell 1993).

Regression analysis

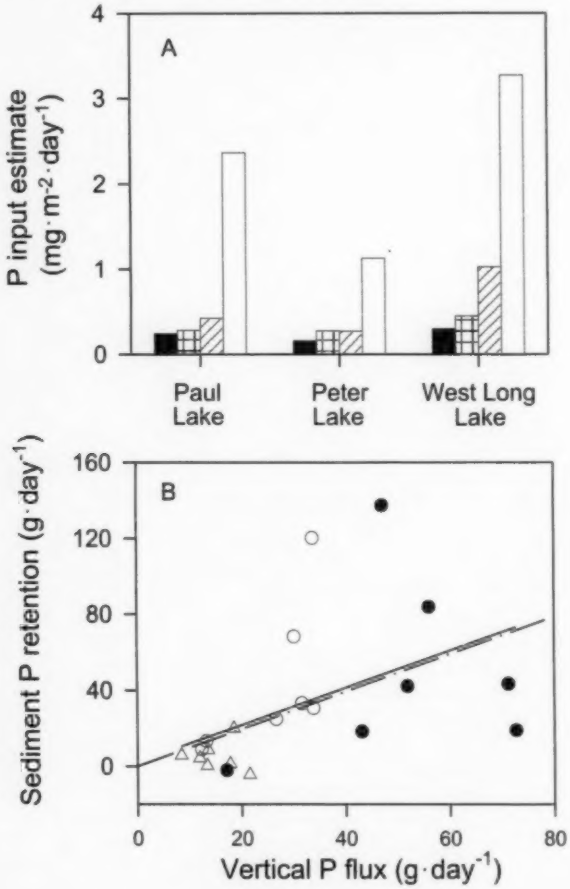
The whole-lake manipulations created a gradient of P inputs and zooplankton grazing that made it possible to use multiple regression to examine the data for significant predictors of vertical P flux. To correct for autocorrelation in the residuals, an autoregressive lag-1 model was used. Normal probability plots suggested that the residuals were approximately normal.

Results

Sediment focusing

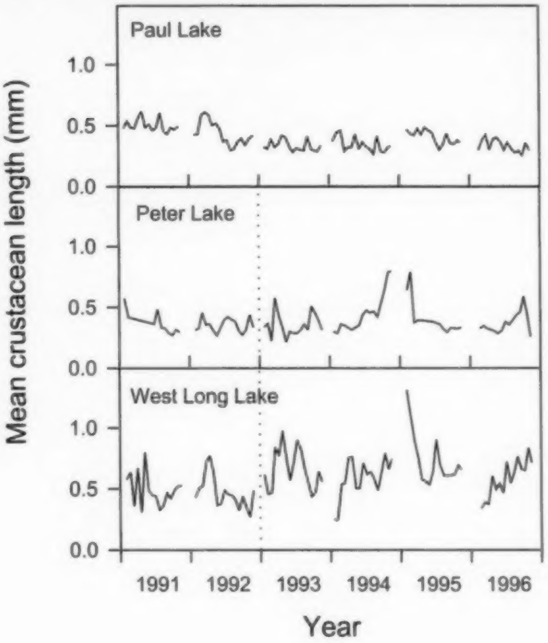
In Peter and West Long lakes, the maximum sediment accumulation rate and maximum ^{210}Pb inventory occurred in the sediment cores from the central, deeper regions of the lakes (Table 2). Thus, sediment focusing is a significant process in these lakes. Using the sediment core data, the whole-lake ^{210}Pb inventory was estimated to be 23.0 dpm·cm^{-2} in Peter Lake and 37.5 dpm·cm^{-2} in West Long Lake. Crusius and Anderson (1995) calculated that the atmospheric input of ^{210}Pb of approximately 1 dpm·cm^{-2} to lakes in this region would yield a steady-state ^{210}Pb inventory of between 16 and 32 dpm·cm^{-2}. Our calculated ^{210}Pb inventories are corroborated by these estimates. In Peter and West Long lakes, the ratio of the ^{210}Pb inventory (dpm·cm^{-2}) of the deep central core to the whole-lake inventory was used to estimate the magnitude of sediment focusing. In both Peter and West Long lakes, the deepest core ^{210}Pb inventory is approximately three times the estimated whole-lake inventory, indicating that for these lakes, whole-lake sediment accumulation rates are approximately one third the sediment accumulation rates from the deep central zone of the lake (Table 2). Because Peter and West Long lakes had similar focusing (Table 2), and the

Fig. 2. Evaluation of sediment trap measurements by (A) comparing estimates of premanipulation P inputs to Paul, Peter, and West Long lakes based on sediment cores (solid bars), a P loading equation (Vollenweider 1976) (horizontally and vertically cross-hatched bars), focus-corrected sediment traps (diagonally hatched bars), and uncorrected sediment traps (open bars) and (B) comparison of sediment P retention calculated by mass balance and vertical P flux measured by sediment traps for Paul (triangles), Peter (open circles), and West Long (solid circles) lakes. Both the 1:1 line (dotted–dashed) and regression line (solid) are shown. Regression equation: retention = –0.194 + 0.995 × vertical P flux; $R^2 = 0.25$, $p = 0.02$.



morphometry of all three lakes is similar (Table 1), we assumed that the sediment focusing in Paul Lake could be approximated as the mean of the focusing in Peter and West Long lakes. Sediment trap measurements of vertical P flux were corrected according to these estimates of focusing. Because the correction for focusing is similar among lakes, the correction has little effect on the among-lake comparisons but is important for obtaining estimates of vertical P flux for use in lake P budgets.

Fig. 3. Mean crustacean length time series for the experimental lakes. The vertical dotted line indicates when enrichment began.



Sediment traps

To demonstrate that the focus-corrected sediment traps were providing reasonable estimates of seasonal sediment P retention, preenrichment (1991–1992) P inputs to each lake were estimated by difference using the corrected sediment trap estimates of the vertical P flux and sediment core estimates of long-term sediment P retention. These P input estimates were compared with estimates of P input based on Vollenweider's (1976) P loading equation. There is reasonable agreement between the P input estimates based on sediment cores, corrected sediment traps, and the P loading equation (Fig. 2A). Furthermore, a comparison of focus-corrected sediment trap measurements of vertical P flux and seasonal sediment P retention calculated by mass balance shows no systematic differences (Fig. 2B). Focus-corrected sediment trap measurements of vertical P flux were used as a surrogate measure of sediment P retention to test our hypotheses concerning the relationship of sediment P retention to other variates.

Zooplankton

The abundance of planktivorous minnows in Peter Lake maintained a zooplankton community consisting of relatively small zooplankton. The zooplankton community was dominated by *Bosmina* spp. (mean length (±SD) = 0.33 ± 0.067 mm), small copepods, rotifers, and occasionally *D. rosea* (mean length = 0.91 ± 0.21 mm). The mean crustacean length in Peter was relatively small, ranging from 0.27 to 0.79 mm (Fig. 3).

A wider range of mean crustacean lengths occurred in piscivore-dominated West Long Lake where mean crustacean length varied from 0.31 to 1.09 mm (Fig. 3). The most abundant zooplankton were *D. pulex* (mean length = 1.2 ± 0.22 mm) and *D. rosea* (mean length = 1.1 ± 0.24 mm),

Fig. 4. Biweekly data show the positive correlation ($r = 0.71$) between mean crustacean length and vertical P flux for Paul (triangles), Peter (open circles), and West Long (solid circles) lakes.

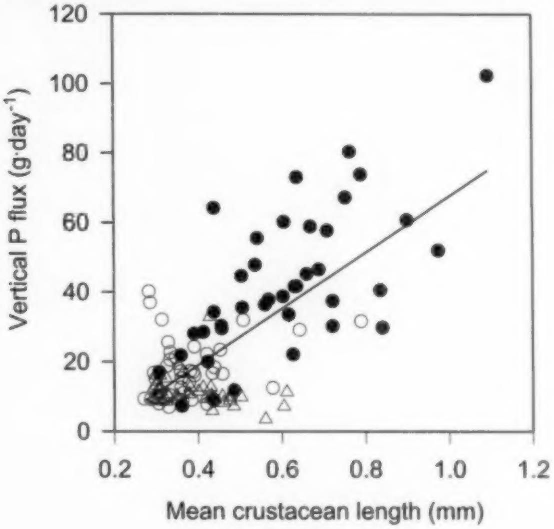
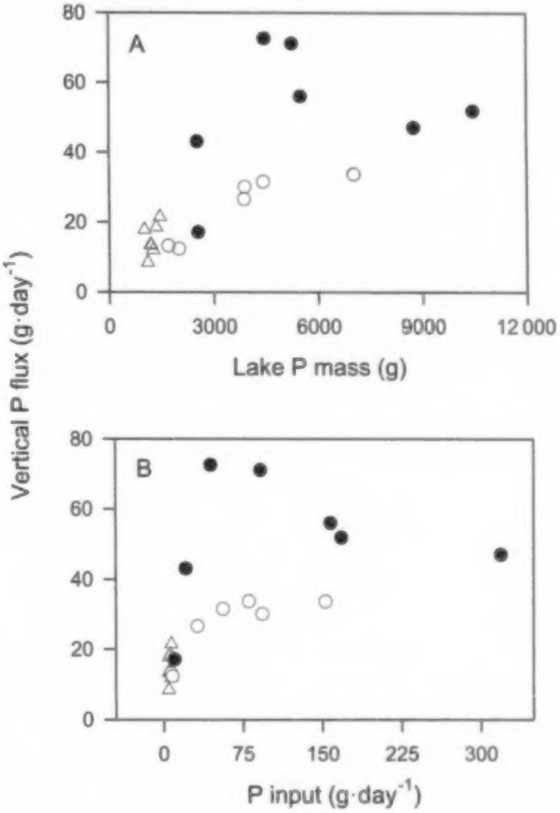


Fig. 5. Positive correlation between the vertical P flux and (A) the mass of P in the lake ($r = 0.69$) and (B) the P input ($r = 0.58$) for Paul (control lake, triangles), Peter (nutrient enriched, open circles), and West Long (nutrient enriched, solid circles) lakes. Data shown are seasonal averages.



which comprised 35 and 24% of the biomass, respectively (see Pace et al. (1998) for more detailed description of the zooplankton communities).

Vertical P flux

Biweekly data from Peter and West Long lakes were examined separately for patterns in vertical P flux within lakes using linear regression. Paul Lake, the reference lake, experienced little variation in P input rate, P mass in the water column, mean crustacean length, or vertical P flux; linear regression would not be expected to find any of the independent variables to be significant predictors of vertical P flux.

As indicated by the range of mean crustacean length, Peter Lake experienced a more limited range of algal grazing intensity by zooplankton than West Long Lake (Fig. 3). In Peter Lake, the significant terms in the linear regression model (df = 38) for vertical P flux (grams per day) were an autoregressive term and either P mass in the water column (0.0016 ± 0.00071 g; $R^2 = 0.276$, $p = 0.033$) or P loading rate (0.059 ± 0.023 g·day^{-1}; $R^2 = 0.302$, $p = 0.016$). Mean crustacean length was not a significant predictor of vertical P flux in Peter Lake ($p = 0.265$). However, in West Long Lake, which experienced a wide range of zooplankton grazing intensity, neither P mass in the water column ($p = 0.44$) nor P loading rate ($p = 0.68$) was significant when regressed against vertical P flux; the best linear regression model (df = 38) contained only an autoregressive term and mean crustacean length (49.84 ± 12.71 mm; $R^2 = 0.529$, $p = 0.0004$).

Biweekly data from Paul (the reference lake), Peter, and West Long lakes were combined and regression analysis used to detect significant predictors of vertical P flux in the pooled data. As individual predictor variables, crustacean length ($R^2 = 0.76$, $p = 0.0001$), lake P mass ($R^2 = 0.71$, $p = 0.012$), and P input ($R^2 = 0.71$, $p = 0.023$) were significant predictors of vertical P flux (Figs. 4 and 5). Changes in lake P mass and P input are highly correlated (Fig. 6); inclusion of both terms in the multiple regression would be redundant. Lake P mass was chosen for inclusion in the model because it was always measured directly. Mean crustacean length was not well correlated with P input ($r = 0.29$) or lake P mass ($r = 0.19$). Both mean crustacean length and lake P mass were significant predictor variables in the multiple regression analysis (Table 3).

Phosphorus accumulation in the water column

The preenrichment P retention of the lakes ($R_P = (I_P - O_P)/I_P$) ranged from 0.75 to 0.86. During enrichment, P retention of the lakes increased and ranged from 0.91 to 0.98. This increase in P retention was due in part to the accumulation of P in the water column of the enriched lakes during enrichment (Fig. 7). No trends in P standing stock of Paul, the reference lake, were seen (Fig. 7). The water column accumulation of the enriched lakes was relatively low during the first year of enrichment (7% of the P input in Peter Lake and 32% in West Long Lake for 1993) and rose substantially over the course of enrichment (62% of P input in Peter Lake and 41% in West Long Lake for 1997). Most of the P accu-

Fig. 6. Positive correlation between P input and change in lake P mass ($r = 0.88$) for Paul (triangles), Peter (open circles), and West Long (solid circles) lakes. The dotted–dashed line is the 1:1 line and the solid line is the regression line.

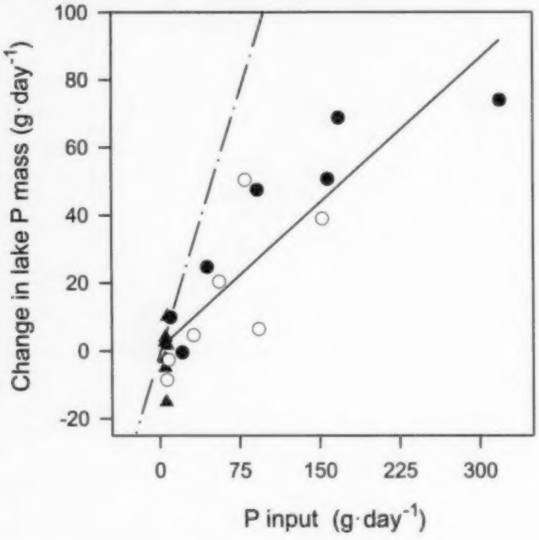
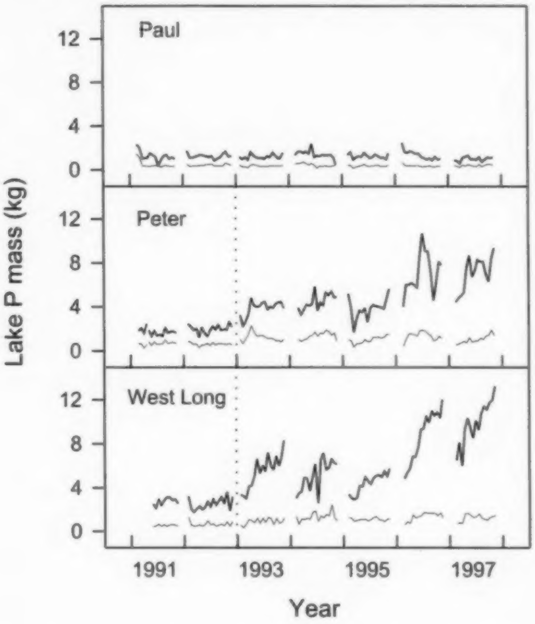


Fig. 7. Mass of P in the experimental lakes for 1991–1997. The vertical dotted line shows the beginning of the experimental P enrichment. The thick line represents the entire water column P mass, and the thin line represents that of the epilimnion; the difference between the two is the P mass in the hypolimnion.



mulation occurred in the hypolimnion (Fig. 7), and the P accumulation in the lakes was correlated with the TP input to the lake (Fig. 6). Phosphorus standing stock increased steadily during the stratified period of each enrichment year but returned to pretreatment levels in the springs of 1994 and 1995 (Fig. 7). Beginning in 1996, West Long Lake failed to return to preenrichment levels over the winter.

Mean epilimnetic P concentrations increased with P input, but large accumulations of P in the epilimnion did not occur (Fig. 7). The P concentrations in the epilimnetic waters, while two to three times higher than preenrichment concentrations, were low compared with the amount of P added to the lake. The change in epilimnetic P standing stock was never greater than 25 $mg \cdot m^{-2}$ (approximately 1.0 kg) during stratification, while as much as 600 $mg \cdot m^{-2}$ (approximately 30 kg) was added the lakes.

Discussion

We maintained contrasting food webs in three experimental lakes for 6 years and manipulated the nutrient input rate over a wide range. Our results show that over 6 years and three lakes, and over a large range of nutrient loading rates, the presence of abundant large zooplankton grazers (as indicated by increased mean crustacean length) was consistently associated with increased vertical P flux. In fact, in West Long Lake, mean crustacean length was the only significant variable in the regression equation for vertical P flux.

In previous enclosure experiments that directly manipulated zooplankton, those that were fertilized (Uehlinger and Bloesch 1987; Bloesch and Bürgi 1989) showed positive effects of zooplankton on sedimentation, while those that were not fertilized showed a negative effect (Bürgi et al. 1979; Sarnelle 1992, 1999). In fertilized enclosures, Bloesch and Bürgi (1989) found that the presence of herbivorous zooplankton, through aggregation of particles in fecal material and the sinking of dead zooplankton, increased PP vertical flux 25–57%, and Uehlinger and Bloesch (1987) found that limnocorrals from which herbivorous zooplankton had been removed had lower vertical P flux. In contrast, Bürgi et al. (1979) and Sarnelle (1992, 1999) found that in unfertilized enclosure experiments, abundant herbivorous zooplankton reduced sedimentation. Enclosure experiments in which fish were manipulated causing differences in zooplankton communities produced similar results (Langeland et al. 1987; Mazumder et al. 1989). Our results from experimentally fertilized lakes concur with this pattern.

Previous studies on whole lakes showed that following the removal of fish and subsequent proliferation of large crustaceans, vertical P flux was reduced due to the reduction in particle concentration in the water column (Reinertsen et al. 1990; Sarnelle 1992) or that the effect of zooplankton biomass on vertical P flux varies between lakes (Elser et al. 1995; but see reanalysis by Sarnelle 1999). Both Sarnelle (1992, 1999) and Reinertsen et al. (1990) reported that P sedimentation was reduced during the dominance of large crustaceans because the large crustaceans were associated with a reduction in the epilimnetic concentration of PP. The fate of this removed PP is not well known but is likely a combination of P accumulation in the zooplankton, increased dissolved P concentrations (Sarnelle 1992), and losses to sedimentation via sinking dead zooplankton (Reinertsen et al. 1990). For example, Reinertsen et al. (1990) attributed the 30% reduction in TP concentration in

Table 3. Summary of vertical P flux (g·day^{-1}) regression analysis ($R^2 = 0.769$).

Dependent variable: Vertical P flux (t)					
Independent variable	Slope ± SE		t		p
Mean crustacean length (t) (mm)	34.620±6.638		5.215		8.60×10^{-7}
Vertical P flux ($t - 1$) (autoregressive term)	0.591±0.064		9.270		1.70×10^{-15}
Lake P mass (t) (g)	0.001±.00043		1.977		0.05
y-intercept	–8.856±2.676		5.353		0.001
ANOVA					
Source	SS	df	MS	F ratio	p
Model	30 792.4	3	10 264.1	121.9	9.99×10^{-16}
Residual	9 262.0	110	84.2		

the lake (which led to a reduction in the bulk P sedimentation) to increased sedimentation of dead zooplankton along with reduced P release from epilimnetic sediments and reduced P excretion from fish. Thus, while these studies found that abundant large crustaceans reduced TP sedimentation rates, the PP settling velocity (PP sedimentation rate divided by euphotic zone PP concentration) increased in the presence of abundant *Daphnia*. In contrast with previous whole-lake studies, we found abundant large herbivorous zooplankton to be associated with increased vertical P flux. It has been suggested that this positive effect of zooplankton on vertical P flux is more likely in oligotrophic lakes than in mesotrophic or eutrophic lakes (Sarnelle 1999). However, West Long Lake is a mesotrophic to eutrophic lake, yet showed a positive correlation between vertical P flux and mean crustacean length.

In contrast with previous enclosure and whole-lake studies that manipulated fish such that the treatments were the presence or absence of fish and observed changes in zooplankton populations and sedimentation, the contrasting zooplankton communities in our study lakes were maintained by the presence of either planktivorous or piscivorous fish. By maintaining contrasting fish communities in Peter (planktivore) and West Long (piscivore) lakes, we were able to evaluate the effects of these contrasting food webs on vertical P flux in the presence of variable P input rates. Enclosure experiments have shown that the presence or absence of fish, in addition to shaping the zooplankton community, can directly affect the phytoplankton (Vanni and Layne 1997), and therefore sedimentation rate, complicating the interpretation of zooplankton effects in experiments that manipulate fish populations (Sarnelle 1999). We cannot separate the between-lake effects of the fish populations from those of the zooplankton populations, and the interactions between fish populations, nutrient loading, and mean crustacean length may be complex (Sarnelle 1999). However, we do show a strong correlation between mean crustacean length and P sedimentation rate within piscivore-dominated West Long Lake.

Our results, along with the published enclosure studies, suggest that, depending on the relative rates of sedimentation of phytoplankton and zooplankton feces, abundant large crustaceans, through particle aggregation and sinking of dead zooplankton, can increase vertical P flux. We speculate that the experimental nutrient enrichment of our lakes prevented the PP supply of the epilimnion from being completely depleted and abundant large-bodied crustaceans maintained a high rate of vertical P flux. Predictions of vertical P flux may be improved by including a term such as mean crustacean length to characterize the food web of a lake.

Phosphorus dynamics during nutrient enrichment

The increase in vertical P flux during nutrient enrichment was insufficient to prevent P accumulation in the water column. Clearly, the increase in sediment P retention was less than the increase in P inputs. The years with the largest P accumulation in the water column followed years of relatively high P input to the lakes. Phosphorus release from the sediments in 1994 and 1997 may have reduced sediment P retention, leading to larger P accumulation in the water column.

Sas (1989) found that, in shallow lakes, sediments frequently were net sources of P following reduction in external P inputs as a part of lake restoration efforts. In our study lakes, the years 1994 and 1997 are analogous to the onset of restoration because the P enrichment was considerably lower in these years than in the preceding years. Furthermore, the surficial sediment P concentration in our lakes was as high as 3.6 mg P·g dry sediment^{-1}, well above the threshold of 1 mg P·g dry sediment^{-1} that Sas (1989) proposed for the occurrence of P release from sediments.

In other experimentally enriched lakes, sediment P retention was high compared with our lakes, and much of the added P settled quickly and permanently (Einsele 1941, cited in Hutchinson 1957, p. 745; Schindler et al. 1987). Schindler et al. (1987) found the average increase in P in the water column in Lake 227 (Experimental Lakes Area (ELA), Ontario, Canada) to be approximately 1–2% of the P input, and sediment retention accounted for 89–91% of the P input. By the final year of enrichment, water column accumulation in our lakes during the field season was 41% of the P input in West Long Lake and 63% in Peter Lake.

Lake 227 is similar to the UNDERC lakes in both oxygen dynamics and important chemical aspects. Lake 227 and our study lakes both have anoxic hypolimnia for much of the year and have similar iron and sulfate concentrations (J.N. Houser and J.J. Cole, unpublished data; Stross and Hasler 1960; Schindler 1985; Schindler et al. 1987). At both ELA and UNDERC, sulfate concentrations are low enough to lead to low sediment P release (i.e., high sediment retention) under both oxic and anoxic conditions (Caraco et al. 1989). ELA lakes exhibit the low sediment P release expected to correspond to low sulfate concentrations but UNDERC lakes do not. A major difference between the two studies is that the P additions to Lake 227 were approximately five times the natural P input, while we added up to 20 times the natural P input to the UNDERC lakes. These high P input rates may have exhausted the capacity of the sediments for further net P sorption, allowing P release into the overlying water.

While P accumulation in the water column could be high during the summer stratified season, P levels in the enriched lakes returned to preenrichment levels during the winter during the first 3 years of enrichment. However, in 1996 and 1997, the P concentrations in West Long Lake were still elevated at the beginning of the field season from the previous nutrient additions. The large amount of P that accumulated in the water column in West Long Lake in 1996 and 1997 and the elevated P concentrations at the beginning of subsequent field seasons indicate that sediment P retention became less effective in removing P from the water column as enrichment continued. Similar changes have been noted in lakes that have received heavy P inputs for extended periods of time (Armengol et al. 1986).

Our results corroborate studies that have found sediment traps to overestimate vertical P flux relative to sediment P retention measured by mass balance (Dillon et al. 1990) or sediment cores (Davis 1968; Bloesch and Evans 1982). However, when corrected for sediment focusing, sediment trap measurements of vertical P flux can provide useful information about seasonal sediment P retention.

The effect of food web structure on vertical P flux and the decrease in the proportion of P input retained in the sediments as P input rate increased indicate that predicting sediment P retention as a constant proportion of input has important limitations. The use of P loading models to accurately predict lake P concentration may be limited by neglect of food web effects and the variability of the proportion of P input that is retained in the sediments.

Acknowledgments

We thank T. Essington, B. Foreman, J. Kitchell, T. Reed, and two anonymous referees for helpful reviews of the manuscript, P. Leavitt for coring lessons, G. Lee for building the sediment corer, and D. Bade, P. Carpenter, T. Essington, B. Herwig, J. Klug, C. Lamon, C. O'Reilly, C. Scheele, and N. Voichick for field and laboratory assistance. This research is a contribution from UNDERC. Financial support was provided by the National Science Foundation and the A.W. Mellon Foundation.

References

Appleby, P.G., Oldfield, F., Thompson, R., Huttunen, P., and Tolonen, K. 1979. ^{210}Pb dating of annually laminated sediments from Finland. Nature (Lond.), **280**: 53–55.

Armengol, J., Crespo, M., Morgui, J.A., and Vidal, A. 1986. Phosphorus budgets and forms of phosphorus in the Sau reservoir sediment: an interpretation of the limnological record. Hydrobiologia, **143**: 331–336.

Bloesch, J. 1994. A review of methods used to measure sediment resuspension. Hydrobiologia, **284**: 13–18.

Bloesch, J., and Bürgi, H.R. 1989. Changes in phytoplankton and zooplankton biomass and composition reflected by sedimentation. Limnol. Oceanogr. **34**: 1048–1061.

Bloesch, J., and Burns, N.M. 1980. A critical review of sedimentation trap technique. Schweiz. Z. Hydrol. **42**: 15–55.

Bloesch, J., and Evans, R.D. 1982. Lead-210 dating of sediments compared with accumulation rates estimated by natural markers and measured with sediment traps. Hydrobiologia, **92**: 579–586.

Bürgi, V.H.R., Buhrer, H., Bloesch, J., and Szabo, E. 1979. Der Einfluss experimentell variierter Zooplanktondichte auf di Produktion und Sedimentation im hocheutrophen See. Schweiz. Z. Hydrol. **41**: 38–63.

Canfield, D.E., and Bachmann, R.W. 1981. Prediction of total phosphorus concentrations, chlorophyll *a*, and secchi depths in natural and artificial lakes. Can. J. Fish. Aquat. Sci. **38**: 414–423.

Caraco, N.F., Cole, J.J., and Likens, G.E. 1989. Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. Nature (Lond.), **341**: 316–318.

Carpenter, S.R., and Kitchell, J.F. (*Editors*). 1993. The trophic cascade in lakes. Cambridge University Press, Cambridge.

Carpenter, S.R., Kitchell, J.F., Cottingham, K.L., Schindler, D.E., Christensen, D.L., Post, D.M., and Voichick, N. 1996. Chlorophyll variability, nutrient input, and grazing: evidence from whole-lake experiments. Ecology, **77**: 725–735.

Cole, J.J., and Pace, M.L. 1998. Hydrologic variability of small, northern Michigan lakes measured by the addition of tracers. Ecosystems, **1**: 310–320.

Cross, P.M., and Rigler, F.H. 1983. Phosphorus and iron retention in sediments measured by mass budget calculations and directly. Can. J. Fish. Aquat. Sci. **40**: 1589–1597.

Crusius, J., and Anderson, R.F. 1995. Sediment focusing in six small lakes inferred from radionuclide profiles. J. Paleolimnol. **13**: 143–155.

Davis, M.B. 1968. Pollen grains in lake sediments: redeposition caused by seasonal water circulation. Science (Washington, D.C.), **62**: 796–799.

Dillon, P.J., and Evans, H.E. 1993. A comparison of phosphorus retention in lakes determined from mass balance and sediment core calculations. Water Res. **27**: 659–668.

Dillon, P.J., and Rigler, F.H. 1974. A test of a simple nutrient budget model predicting the phosphorus concentration in lake water. J. Fish. Res. Board Can. **31**: 1771–1778.

Dillon, P.J., Evans, R.D., and Molot, L.A. 1990. Retention and resuspension of phosphorus, nitrogen, and iron in a central Ontario lake. Can. J. Fish. Aquat. Sci. **47**: 1269–1274.

Dymond, J., Collier, R., McManus, J., and Larson, G.L. 1996. Unbalanced particle flux budgets in Crater Lake, Oregon: implications for edge effects and sediment focusing in lakes. Limnol. Oceanogr. **41**: 732–743.

Elser, J.J., Foster, D.K., and Hecky, R.E. 1995. Effects of zooplankton on sedimentation in pelagic ecosystems: theory and test in two lakes of the Canadian shield. Biogeochemistry, **30**: 143–170.

Evans, R.D., and Rigler, F.H. 1980. Measurement of whole lake sediment accumulation and phosphorus retention using lead-210 dating. Can. J. Fish. Aquat. Sci. **37**: 817–822.

Hilton, J. 1985. A conceptual framework for predicting the occurrence of sediment focusing and sediment redistribution in small lakes. Limnol. Oceanogr. **30**: 1131–1143.

Houser, J.N. 1998. Food web structure and experimental enrichment: effects on phosphorus sedimentation and retention. M.Sc. thesis, University of Wisconsin-Madison, Madison, Wis.

Hutchinson, G.E. 1957. A treatise on limnology. Vol. 1. John Wiley & Sons, Inc., New York.

Langeland, A., Koksvik, J.I., Olsen, Y., and Reinertsen, H. 1987. Limnocorral experiments in a eutrophic lake — effects of fish on the planktonic and chemical conditions. Pol. Arch. Hydrobiol. **34**: 51–65.

Likens, G.E., and Davis, M.B. 1975. Post-glacial history of Mirror Lake and its watershed in New Hampshire, U.S.A., an initial report. Int. Ver. Theor. Angew. Limnol. Verh. **19**: 982–993.

Mazumder, A., Taylor, W.D., McQueen, D.J., and Lean, D.R.S. 1989. Effects of fertilization and planktivorous fish on epilimnetic phosphorus and phosphorus sedimentation in large enclosures. Can. J. Fish. Aquat. Sci. **46**: 1735–1742.

Pace, M.L. 1984. Zooplankton community structure, but not biomass, influences the phosphorus – chlorophyll *a* relationship. Can. J. Fish. Aquat. Sci. **41**: 1089–1096.

Pace, M.L., Cole, J.J., and Carpenter, S.R. 1998. Trophic cascades and compensation: differential responses of microzooplankton in whole-lake experiments. Ecology, **79**: 138–152.

Paffenhöfer, G.A.,and Knowles, S.C. 1979. Ecological implications of fecal pellet size, production and consumption by copepods. J. Mar. Res. **37**: 35–49.

Prairie, Y.T. 1988. A test of the sedimentation assumptions of phosphorus input–output models. Arch. Hydrobiol. **111**: 321–327.

Reinertsen, H., Jensen, A., Koksvik, J.I., Langeland, A., and Olsen, Y. 1990. Effects of fish removal on the limnetic ecosystem of a eutrophic lake. Can. J. Fish. Aquat. Sci. **47**: 166–173.

Robbins, J.A. 1978. Geochemical and geophysical applications of radioactive lead. *In* The biogeochemistry of lead in the environment. *Edited by* J.O. Nriagu. Elservier/North-Holland Biomedical Press, New York. pp. 285–405.

Rowan, D.J., Cornett, R.J., King, K.J., and Risto, B.A. 1995. Sediment focusing and ^{210}Pb dating: a new approach. J. Paleolimnol. **13**: 107–118.

Sarnelle, O. 1992. Contrasting effects of *Daphnia* on ratios of nitrogen to phosphorus in a eutrophic, hard-water lake. Limnol. Oceanogr. **37**: 1527–1542.

Sarnelle, O. 1999. Zooplankton effects on vertical particulate flux: testable models and experimental results. Limnol. Oceanogr. **44**: 357–370.

Sas, H. (*Editor*). 1989. Lake restoration by reduction of nutrient loading: expectations, experiences, extrapolations. Academia-Verlag Richarz, Sankt Augustin.

Schindler, D.E., Kitchell, J.F., He, X., Carpenter, S.R., Hodgson, J.R., and Cottingham, K.L. 1993. Food web structure and phosphorus cycling in lakes. Trans. Am. Fish. Soc. **122**: 756–772.

Schindler, D.W. 1985. The coupling of elemental cycles by organisms: evidence from whole-lake chemical perturbations. *In* Chemical processes in lakes. *Edited by* W. Stumm. John Wiley & Sons, Inc., New York. pp. 225–250.

Schindler, D.W., Hesslein, R.H., and Turner, M.A. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. **44**(Suppl. 1): 26–33.

Stross, R.G., and Hasler, A.D. 1960. Some lime-induced changes in lake metabolism. Limnol. Oceanogr. **5**: 265–272.

Swain, A.M. 1973. A history of fire and vegetation in northeastern Minnesota as recorded in lake sediments. Quat. Res. **3**: 383–396.

Uehlinger, U., and Bloesch, J. 1987. The influence of crustacean zooplankton on the size structure of algal biomass and suspended and settling seston (biomanipulation in limnocorrals II). Int. Rev. Gesamten Hydrobiol. **72**: 473–486.

Vanni, M.J., and Layne, C.D. 1997. Nutrient recycling and herbivory as mechanisms in the "top-down" effect of fish on algae in lakes. Ecology, **78**: 21–40.

Vollenweider, R.A. 1976. Advances in defining critical loading levels for P in lake eutrophication. Mem. Ist. Ital. Idrobiol. **33**: 53–83.

DISCUSSION

Significance of larval condition: comment on laboratory experiments[1]

Iain Suthers

The significance of condition in larval fish for evaluating many hypotheses and processes in fisheries oceanography is uncertain (Ferron and Leggett 1994; Leggett and DeBlois 1994). Larvae in better condition are presumably less likely to die of starvation or predation on average and thus contribute proportionally more to the numbers at recruitment. Larval condition (or health) may be determined by a variety of biochemical indices (e.g., RNA/DNA ratios, relative lipid content), histological indices, or otolith growth indices. Tests of the growth/condition–mortality hypothesis are typically at two extremes of temporal scales. At the largest scale, condition of wild caught larvae is compared with subsequent recruitment, but the comparison is made over only 2–5 years (e.g., Setzler-Hamilton et al. 1987; Bailey et al. 1995; Ralston and Howard 1995; Campana 1996). At the other extreme, there are laboratory experiments that place larvae of variable condition in the presence of a predator and monitor the condition of the survivors (e.g., McCormick and Kerrigan 1996). There are intermediate spatial scale studies of larval condition (e.g., Buckley and Lough 1987; Suthers 1996; Lochmann et al. 1997), but these do not explicitly test the growth/condition–mortality hypothesis.

A recent article by Elliott and Leggett (1998) experimentally evaluated the hypothesis that fish larvae in poor nutritional condition experienced higher mortality rates due to predation. The authors concluded that the RNA/DNA ratio was not a reliable predictor of survival probability and that the ratio and dry weight were probably of limited use in prorating larval abundance as a predictor of recruitment. This conclusion overstates what can be drawn from their data and illustrates a more general problem in extending the results of laboratory experiments of larval condition to the field. My intention is to illustrate some pitfalls in this area and to offer some solutions to the definition of "larval condition" and how to test its significance.

The experimental arena of Elliott and Leggett (1998) consisted of a 38-L black plastic container containing 75 starved and 75 fed capelin (*Mallotus villosus*) larvae in the presence of a starved juvenile lumpfish (*Cyclopterus lumpus*) of around 30 mm standard length. Each trial lasted about 15 min when 75 larvae had been consumed, which is equivalent to one larva per 6 s. At this rate of removal, the predator is not exercising much discrimination. Additionally, in the field, there would exist planktonic refugia, in the form of floatsom, turbulence, turbidity, and simply extensive space not available in aquaria. Once the larvae left the confines of the beach, the densities of larvae would fall well below the laboratory density of $4 \cdot L^{-1}$, and light levels would be less than that provided by the two fluorescent tubes placed 1 m above the tank. Therefore, reduced light penetration in nature would lead to decreased detection of the larvae and mortality would be reduced as a result, and it may well be more selective.

Laboratory experiments are needed for hypothesis generation and in calibrating condition indices with the effects of capture, preservation, and starvation (e.g., McGurk 1985; Theilacker 1986). Designing ecologically relevant laboratory experiments to test the significance of larval condition is difficult. I suggest that the following be considered. Firstly, "larval condition" needs to be defined, as the term spans a range of time scales and ecological function for different species and stages of larvae (Suthers 1998). For most late-stage larvae, at the scale of weeks, there are morphometric indices (Fulton's *K*) and storage indices (lipid concentration), whereas at the scale of days, there are growth indices (RNA/DNA ratios, otolith growth, cell cycle analysis) or starvation indices (histological status, gut epithelium cell height), and at the scale of hours there are feeding indices (gut fullness, trypsin activity). The different indices are relevant at spatiotemporal scales appropriate to oceanographic phenomena (Fig. 1). Ontogenetic development has a considerable influence on an index, such that the morphometry of early-stage larvae may be sensitive on the order of days (Bisbal and Bengston 1995).

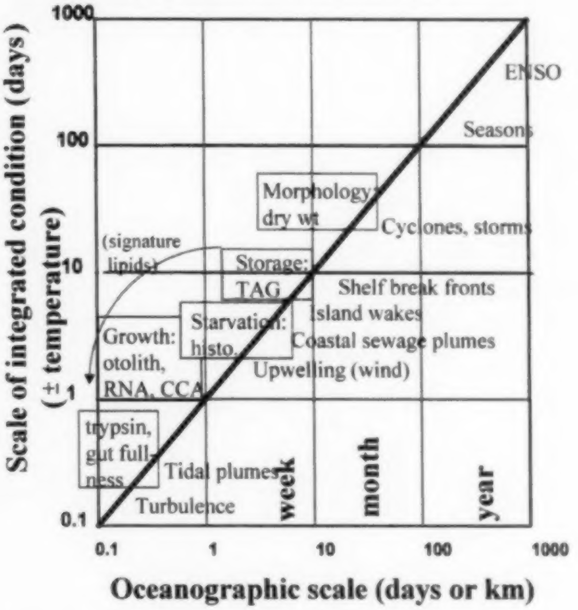
Secondly, as pointed out in Ferron and Leggett (1994), reared larvae (regardless of their condition) tend to be fatter, with less histological variation and bigger hearts, and condition is influenced by the container volume (see references in Suthers 1998). The survival of the reared larvae also needs to be reported, as differential survival (due to stress or dis-

Received September 13, 1999. Accepted January 31, 2000.
J15351

I. Suthers. School of Biological Science, University of New South Wales, Sydney 2052, New South Wales, Australia.
e-mail: i.suthers@unsw.edu.au

[1] Comment on paper by J.K. Elliott and W.C. Leggett. 1998. Larval condition and vulnerability to predation: an analysis based on mixed-prey experiments. Can. J. Fish. Aquat. Sci. **55**: 626–630.

Fig. 1. Summary of the temporal scales of condition indices (within boxes) related to some spatiotemporal oceanographic phenomena for typical late-stage larvae. Morphological indices may be temporally more sensitive in early-stage larval fish. Lipid indices are linked to short-term feeding indices by the use of characteristic phytoplankton-derived signature lipids.



ease) may leave a population of larvae of better condition. In Elliott and Leggett's (1998) study, the combined fed and starved negative growth rates in RNA/DNA ratio and dry weight of larvae (declines of over 30% in RNA/DNA and 15% in dry weight over 4 days; their fig. 2) show that the larvae were not particularly viable. Laboratory-reared, fed larvae may have full guts (as in their study) but for a variety of reasons may not be growing.

Thirdly, the effect of size needs to be removed from an index (and reported in the Materials and methods section), which may not be achieved simply by a ratio (Suthers et al. 1996). Finally, as the mortality rate of larvae in the ocean is effectively 1, it is expected that larvae in good condition will also suffer high mortality rates under certain conditions.

The larval survival process and the significance of larval condition involve other complexities as revealed by modelling (Sclafani et al. 1993) and an analysis of behaviour (Neilson et al. 1986; Chesney 1989) and in situ swimming speeds (Leis and Carson-Ewart 1997). The use of small predator arenas cannot produce ecologically useful conclusions about larval condition indices, and the general recommendation of Elliott and Leggett (1998) that RNA/DNA ratios have limited predictive use is not justified.

Laboratory rearing experiments do have their place for examining relative changes among species and relative ontogenetic changes within species. The growth/condition–mortality hypothesis may be tested under carefully designed and monitored laboratory experiments that match the temporal scale of the index with that of the hypothesis. Predator arenas should be as large as possible (5–10 m^3), with densities of larvae $<1 \cdot L^{-1}$, and should include refugia, with the experiment lasting for a period of days (not minutes). The relevant condition index needs to be justified on ecological grounds, and for many larvae, a growth index is probably the most useful. In fact, the ecological relevance of various condition indices still needs further investigation. At present, large-scale analyses of recruitment still provide the most convincing tests of significance of larval growth/condition–mortality (e.g., Campana 1996). Future studies should consider in situ small-scale spatial and temporal studies that link mortality and condition of cohorts, such as within an estuary. This would provide a rigorous test of the hypothesis and provide managers and scientists alike with a useful tool to investigate the cascade of events leading to annual fluctuations in recruitment.

References

Bailey, K.M., Canino, M.F., Napp, J.M., Spring, S.M., and Brown, A.L. 1995. Contrasting years of prey levels, feeding conditions and mortality of larval walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska. Mar. Ecol. Prog. Ser. **119**: 11–23.

Bisbal, G.A. and Bengston, D.A. 1995. Description of the starving condition in summer flounder, *Paralichthys dentatus*, early life history stages. Fish. Bull. U.S. **93**: 217–230.

Buckey, L.J., and Lough, R.G. 1987. Recent growth, biochemical composition, and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. Can. J. Fish. Aquat. Sci. **44**: 14–25.

Campana, S.E. 1996. Year-class strength and growth rate in young Atlantic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. **135**: 21–26.

Chesney, E.J. 1989. Estimating the food requirements of striped bass larvae *Morone saxatilis*: effects of light, turbidity and turbulence. Mar. Ecol. Prog. Ser. **53**: 191–200.

Elliott, J.K. and Leggett, W.C. 1998. Larval condition and vulnerability to predation: an analysis based on mixed-prey experiments. Can. J. Fish. Aquat. Sci. **55**: 626–630.

Ferron, A., and Leggett, W.C. 1994. An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. **30**: 217–303.

Leggett, W.C., and DeBlois, E. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth. J. Sea Res. **32**: 119–134.

Leis, J.M., and Carson-Ewart, B.M. 1997. In situ swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. Mar. Ecol. Prog. Ser. **159**: 165–174.

Lochmann, S.E., Taggart, C.T., Griffin, D.A., Thompson, K.R., and Maillet, G.L. 1997. Abundance and condition of larval cod (*Gadus morhua*) at a convergent front on Western Bank, Scotian Shelf. Can. J. Fish. Aquat. Sci. **54**: 1461–1479.

McCormick, M.I., and Kerrigan, B.A. 1996. Predation and its influence on the condition of a newly settled tropical demersal fish. Mar. Freshwater Res. **47**: 557–562.

McGurk, M. D. 1985. Multivariate analysis of morphometry and dry weight of Pacific herring larvae. Mar. Biol. **86**: 1–11.

Neilson, J.D., Perry, R.I., Valerio, P., and Waiwood, K.G. 1986. Condition of Atlantic cod *Gadus morhua* larvae after the transition to exogenous feeding: morphometrics, buoyancy and predator avoidance. Mar. Ecol. Prog. Ser. **32**: 229–235.

Ralston, S., and Howard, D.F. 1995. On the development of year-class strength and cohort variability in two northern California rockfishes. Fish. Bull. U.S. **93**: 710–720.

Sclafani, M., Taggart, C.T., and Thompson, K.R. 1993. Condition, buoyancy and the distribution of larval fish: implications for vertical migration and retention. J. Plankton Res. **15**: 413–435.

Setzler-Hamilton, E.M., Wright, D.A., Martin, F.D., Millsaps, C.V., and Whitlow, S. I. 1987. Analysis of nutritional condition and its use in predicting striped bass recruitment: field studies. Am. Fish. Soc. Symp. **2**: 115–128.

Suthers, I.M. 1996. Spatial variability of recent otolith growth and RNA indices in pelagic juvenile *Diaphus kapalae* (Myctophidae): an effect of flow disturbance near an island? Mar. Freshwater Res. **47**: 273–282.

Suthers, I.M. 1998. Bigger? Fatter? Or is faster growth better? Considerations on condition in larval and juvenile coral-reef fish. Aust. J. Ecol. **23**: 265–273

Suthers, I.M., Cleary, J.J., Battaglene, S.C., and Evans, R. 1996. Relative RNA content as a measure of condition in larval and juvenile fish. Mar. Freshwater Res. **47**: 301–307.

Theilacker, G.H. 1986. Starvation-induced mortality of young sea-caught jack mackerel, *Trachurus symmetricus*, determined with histological and morphological methods. Fish. Bull. U.S. **84**: 1–17.

Larval condition and vulnerability to predation: reply to comment by Suthers[1]

Joel K. Elliott and William C. Leggett

Scientists recognize that observations and experiments at a variety of spatial and temporal scales and in a variety of different systems are required to obtain a complete understanding of a particular phenomenon. However, scientists also recognize that an individual study can usually only focus on one particular system at a certain time and place and that many different studies are required to achieve a general conclusion. The objective of our study on larval capelin (*Mallotus villosus*) (Elliott and Leggett 1998) was to provide an experimental test of whether larvae in poor condition had lower relative survival rates than larvae in good condition when exposed to a predator. We employed an experimental design that had been successfully used by a variety of other researchers (Litvak and Leggett 1992; Pepin et al. 1992; Pepin and Shears 1995; Elliott and Leggett 1996) to test selective mortality hypotheses under laboratory conditions in small aquaria. In our abstract, we explicitly stated that the results of our study "suggest that RNA/DNA ratios are not useful indicators of vulnerability to predation for larval capelin." Clearly, we did not intend to have this conclusion be considered a broad statement that applied to all other systems. As in all scientific disciplines, we presented our data with the intention that other researchers would conduct further studies using a variety of experimental systems and protocols to provide further tests that would either support or refute our results. After a variety of studies on this topic had been conducted, we would hope that a metanalysis would provide a more complete understanding of the effects of larval condition on vulnerability to predation. Ultimately, the results of this research could lead to the incorporation of larval condition indices into assessments of the future survival probabilities of larvae into fisheries models.

Unfortunately, in the comment by Suthers, our conclusions have been overstated and interpreted as a definitive rejection of the utility of RNA/DNA ratios and larval dry weight in the study of larval condition. As stated above, our conclusions pertained to larval capelin and only in the context of the experimental conditions employed. Furthermore, in the second to last paragraph of Elliott and Leggett (1998), we explicitly stated that our conclusions are based on our results, some of which are in contrast with previous studies of larval capelin in small enclosures: "This difference in outcome may result from the use of different experimental conditions and predator species or from the possibility that different measures of larval size (length versus dry weight) may not be equivalent in their influence on the vulnerability of larvae to predation." In other words, variation in the species used or experimental protocol could have caused a different result (as has been reported in many other studies of selective mortality). A general conclusion on the influence of larval condition on vulnerability to predation cannot be reached until a variety of other studies are conducted on this topic. However, the only other similar study that has been conducted on this topic (McCormick and Kerrigan 1996) has also found that larvae in poor condition do not have lower survival probabilities than larvae in good condition when exposed to a predator. Thus, at this time, the available empirical evidence supports our general conclusion about the effect of larval condition on vulnerability to predation.

The comment of Suthers also intended to illustrate some pitfalls in extending the results of laboratory experiments of larval condition to the field and to offer some solutions to the definition of "larval condition" and how to test its significance. These issues have been discussed in detail in a variety of review papers on larval fish condition (e.g., Ferron and Leggett 1994; Suthers 1998), so we will not address these comments. However, we will address some of the specific comments brought up by Suthers that pertain to our paper. The first issue is that the individual predators used in our experiments (juvenile lumpfish (*Cyclopterus lumpus*)) were "starved" before being used in experimental trials. In our Materials and methods section, we stated that the predators were "not fed for 24 h" and whether they were actually starved was not determined. Suthers expresses concern that the fast feeding rate of the predator suggests that it is not exercising much discrimination. However, the main objective of our experiment was not to test whether the predator was being selective or not but to determine if larvae of different condition would have different abilities of escape from the predator. The feeding of the predator only provided an opportunity for differential escape to occur. If larvae in poor condition were not able to escape as effectively, we expected that they would be preyed upon more heavily than those larvae in good condition that did have an effective escape response.

Suthers also states that it is difficult to design ecologically relevant laboratory experiments to test the significance of larval condition on survival because of differences in conditions between the laboratory and field (e.g., larval densities,

Received March 31, 2000. Accepted April 26, 2000.
J15691

J.K. Elliott[2] and W.C. Leggett. Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada.

---

[1] Reply to I. Suthers. 2000. Significance of larval condition: comment on laboratory experiments. Can. J. Fish. Aquat. Sci. **57**: 1534–1536.

[2] Author to whom all correspondence should be addressed: Department of Biology, University of Puget Sound, 1500 North Warner, Tacoma, WA 98416-0320, U.S.A. e-mail: jkelliott@ups.edu

light levels, planktonic refugia, turbulence, turbidity). We agree with this statement, and this is one of the reasons for choosing to work on capelin larvae for this study. Most fish larvae are difficult to rear in the laboratory to produce enough individuals that will survive the manipulations necessary to perform experimental tests. Capelin larvae are very abundant and can be easily collected in the field and then brought to the laboratory for rearing. The experimental protocols for using the larvae in predation experiments have been worked out in previous studies (Litvak and Leggett 1992; Pepin et al. 1992; Pepin and Shears 1995; Elliott and Leggett 1996), making them a good species for conducting our study, which required a large number of larvae that could survive being transferred into experimental containers with and without predators.

We also chose to work on capelin because the question that we were asking was relevant to observed patterns in the field, and field conditions could be simulated in a reasonable fashion in the laboratory. When capelin larvae emerge from beaches in mass numbers (up to approximately $1000 \cdot m^{-3}$), individuals vary widely in their condition levels. There is indirect field evidence that capelin larvae that exhibit poor condition have lower survival probabilities than larvae in good condition (Frank and Leggett 1981*a*, 1981*b*, 1982). Emerging larvae are exposed to a variety of predators, such as juvenile lumpfish, which were abundant in the vicinity of capelin spawning beaches, and they readily consumed capelin larvae in the laboratory. Thus, our experimental system and protocols were ecologically relevant and were based on established field observations. It was not our goal to perfectly simulate all of the conditions present in the natural environment, since we were most concerned with controlling as many variables as possible and varying only the condition of the larvae. By feeding one group of larvae and not feeding another group, we produced larvae with a wide range of condition levels to be used in the experiments. The wider the variation in condition of larvae used in a trial, the more likely that we expected to detect a difference in vulnerability between the predator treatment and the control. Larvae were able to effectively escape predators within the space available in the 38-L containers used in our study, so if there was differential escape, we expected to detect it in our trials. We did not choose to use large containers because we wanted to observe the predators and determine when approximately half of the larvae were consumed. Also, we wanted to be able to quickly collect the unconsumed larvae and freeze them individually so that our analyses of RNA/DNA ratios were not affected by degradation of nucleic acids. Additionally, small containers have not been shown to produce results significantly different from those using large containers in selective predation trials with capelin larvae (e.g., Litvak and Leggett 1992; Pepin et al. 1992; Elliott and Leggett 1997).

Suthers' comment concludes with suggestions for spatial and time scales that should be used in laboratory experiments if they are to be extrapolated to field conditions. His suggestion that the temporal and spatial scale of the condition index utilized should match the scale of the hypothesis has been articulated in detail by Ferron and Leggett (1994), and Suthers' recommendations of appropriate temporal and spatial scales to employ are not supported by any referenced research studies on this topic. We agree with Suthers' concluding statements that large-scale analyses of recruitment provide the most convincing tests of the significance of larval growth condition to the survival of a cohort. However, understanding the mechanisms causing observed patterns of differential survival will require controlled experiments and observations at smaller spatial and temporal scales. These studies will likely be conducted most effectively in the laboratory, where appropriate factors can be controlled and only the factors of interest varied according to the question of interest. We look forward to reading future empirical studies on larval fish condition and vulnerability to predation that provide an objective contribution to this important area of research.

References

Elliott, J.K., and Leggett, W.C. 1996. The effect of temperature on predation rates of a fish (*Gasterosteus aculeatus*) and a jellyfish (*Aurelia aurita*) on larval capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. **53**: 1393–1402.

Elliott, J.K., and Leggett, W.C. 1997. Influence of temperature on size-dependent predation by a fish (*Gasterosteus aculeatus*) and a jellyfish (*Aurelia aurita*) on larval capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. **54**: 2759–2766.

Elliott, J.K., and Leggett, W.C. 1998. Larval condition and vulnerability to predation: an analysis based on mixed-prey experiments. Can. J. Fish. Aquat. Sci. **55**: 626–630.

Ferron, A., and Leggett, W.C. 1994. An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. **30**: 217–303.

Frank, K.T., and Leggett, W.C. 1981*a*. Wind regulation of emergence times and early larval survival in capelin (*Mallotus villosus*). Can J. Fish. Aquat. Sci. **38**: 215–223.

Frank, K.T., and Leggett, W.C. 1981*b*. Prediction of egg development and mortality rates in capelin (*Mallotus villosus*) from meteorological, hydrographic, and biological factors. Can. J. Fish. Aquat. Sci. **38**: 1327–1338.

Frank, K.T., and Leggett, W.C. 1982. Environmental regulation of growth rate, efficiency, and swimming performance in larval capelin (*Mallotus villosus*), and its application to the match/mismatch hypothesis. Can. J. Fish. Aquat. Sci. **39**: 691–699.

Litvak, M.K., and Leggett, W.C. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Prog. Ser. **81**: 13–24.

McCormick, M.I., and Kerrigan, B.A. 1996. Predation and its influence on the condition of a newly settled tropical demersal fish. Mar. Freshwater Res. **47**: 557–562.

Pepin, P., and Shears, T.H. 1995. Influence of body size and alternate prey abundance on the risk of predation to fish larvae. Mar. Ecol. Prog. Ser. **128**: 279–285.

Pepin, P., Shears, T.H., and de Lafontaine, Y. 1992. Significance of body size to the interaction between a larval fish (*Mallotus villosus*) and a vertebrate predator (*Gasterosteus aculeatus*). Mar. Ecol. Prog. Ser. **81**: 1–12.

Suthers, I.M. 1998. Bigger? Fatter? Or is faster growth better? Considerations on condition in larval and juvenile coral-reef fish. Aust. J. Ecol. **23**: 265–273.

National Research Council Canada
Conseil national de recherches Canada

Copyright Transfer

ASSIGNMENT OF COPYRIGHT

Ms. No. ____________

To: NATIONAL RESEARCH COUNCIL OF CANADA ("NRC") Research Press

From: ____
____
____
____
(name and address of author signing this form)

This refers to the manuscript entitled: ____
____

written by myself and the following additional author(s): ____
____

for publication in: ____

1. I declare that the list of authors fairly conforms to academic conventions about who should be listed. I declare that the manuscript, or its equivalent, has not been submitted for publication elsewhere. If it is accepted for publication by the National Research Council of Canada, I will ensure that it, or its equivalent, is not submitted for publication elsewhere.

2. I declare that I am (check ONE):

☐ (a)	the sole owner of the copyright relating to the manuscript,	and →	I **assign** to NRC the entire copyright.
☐ (b)	a part owner of the copyright, in conjunction with interests held by co-authors or their employers,	and →	I **assign** to NRC my entire interest in the copyright, and I expect the co-authors to also sign a form like this one.
☐ (c)	an agent of my employer with authority to assign the copyright owned by the employer, who is: ____________________,	and →	I **assign** to NRC, on behalf of my employer, my employer's entire interest in the copyright relating to the manuscript, and I expect the co-authors to also sign a form like this one.
☐ (d)	a public servant in Canada (federal, provincial or territorial) or the United Kingdom, so that the copyright is owned by Her Majesty,	and →	I have obtained the approval of my employer, according to the terms of my employment, to grant NRC **non-exclusive permission** to publish the manuscript and administer the copyright.
☐ (e)	a public servant of the United States of America, and subject to the law that creates no U.S. copyright for the manuscript,	and →	I **assign** to NRC, on behalf of my employer, my employer's entire interest in any copyright that may exist outside the U.S., and I expect the co-authors to sign a form like this one.

3. I understand that my submission of this document does not guarantee publication, and that publication will not occur until **all authors** have provided a copyright transfer. If the manuscript is not published within 2 years after I sign this, the assignment is revoked.

Signed at ________________________________ on ____________________
(City and Province/State) (Date)

____
(Signature)

[Standard NRC form; alterations not acceptable.]

Comments on Copyright Transfer
(with extracts from the *Copyright Act*, Revised Statutes of Canada 1985, chapter C-42, as amended)

The publisher is not permitted to publish the manuscript without permission or an assignment of ownership of copyright. For greatest flexibility, and to be prepared to deal with future uses of the published material, the publisher requires ownership.

The owner may assign the right, but that assignment must be in writing:

13.(4) *The owner of the copyright in any work may assign the right, either wholly or partially, and either generally or subject to territorial limitations, and either for the whole term of the copyright or for any other part thereof, and may grant any interest in the right by licence, but no assignment or grant is valid unless it is in writing signed by the owner of the right in respect of which the assignment or grant is made, or by his duly authorized agent.*

The owner may be the author, but in many cases, the owner is the author's employer:

13.(3) *Where the author of a work was in the employment of some other person under a contract of service or apprenticeship and the work was made in the course of his employment by that person, the person by whom the author was employed shall, in the absence of any agreement to the contrary, be the first owner of the copyright, but where the work is an article or other contribution to a newspaper, magazine or similar periodical, there shall, in the absence of any agreement to the contrary, be deemed to be reserved to the author a right to restrain the publication of the work, otherwise than as part of a newspaper, magazine or similar periodical.*

The only exception to taking ownership arises when the manuscript is covered by Crown copyright owned by a Canadian government.

12. *Without prejudice to any rights or privileges of the Crown, where any work is, or has been, prepared or published by or under the direction or control of Her Majesty or any government department, the copyright in the work shall, subject to any agreement with the author, belong to Her Majesty and in that case shall continue for the remainder of the calendar year of the first publication of the work and for a period of fifty years following the end of that calendar year.*

Governments, and in particular the federal government, do not have a simple mechanism for assigning ownership of this particular type of Crown asset. Therefore, when an author is a public servant so that there is Crown copyright in the manuscript, a simple permission to use is acceptable to the publisher, if it is granted by a person with appropriate authority.

Conseil national de recherches
Canada

National Research Council
Canada

Cession du droit d'auteur

CESSION DU DROIT D'AUTEUR

Document n° ________

Au : CONSEIL NATIONAL DE RECHERCHES DU CANADA («CNRC») - Presses scientifiques

De : ______________________________

____

____

____

(nom et adresse de l'auteur qui signe le présent formulaire)

La présente cession porte sur le document intitulé : ______________________________

____

rédigé par moi, en collaboration avec l'auteur/les auteurs suivant(s) : ______________________________

____

aux fins de publication dans : ______________________________

1. Je déclare que la liste des auteurs ci-dessus respecte les pratiques usuelles de rédaction savante quant aux personnes dont le nom doit être cité. Je déclare que le document ou son équivalent n'a pas été soumis à quelque autre organisme aux fins de publication. Si le CNRC accepte de publier le document, je m'assurerai que celui-ci ou son équivalent n'est soumis à aucun autre organisme aux fins de publication sans en obtenir la permission préalable du CNRC.

2. Je déclare être (UN SEUL choix) :

☐	(a)	le seul titulaire de droit d'auteur afférent au document,	et →	je **cède** le droit d'auteur intégral au CNRC.
☐	(b)	un titulaire partite du droit d'auteur, de concert avec des coauteurs ou leurs employeurs,	et →	je **cède** ma part du droit d'auteur au CNRC et m'attends à ce que les coauteurs signent une entente comparable.
☐	(c)	un agent de mon employeur ayant l'autorité de céder le droit d'auteur appartenant à l'employeur, nommément : ______________________,	et →	je **cède** au CNRC, au nom de mon employeur, la part entière du droit d'auteur afférent au document appartenant à mon employeur, et m'attends à ce que les coauteurs signent une entente comparable.
☐	(d)	un fonctionnaire du Canada (fédéral, provincial ou territorial) ou du Royaume-Uni, donc le droit d'auteur appartient à Sa Majesté,	et →	j'ai obtenu de mon employeur, en vertu de mes conditions d'emploi, l'autorisation d'accorder au CNRC la **permission non exclusive** de publier le document et de gérer le droit d'auteur.
☐	(e)	un fonctionnaire des États-Unis d'Amérique, donc sujet à la loi qui n'associe aucun droit d'auteur américain au document,	et →	je **cède** au CNRC, au nom de mon employeur, la part entière de quelque droit d'auteur en vigueur ailleurs qu'aux É.-U. appartenant à mon employeur, et m'attends à ce que les coauteurs signent une entente comparable.

3. Je comprends que la soumission du document ne garantit aucunement sa publication et que la publication ne pourra être réalisée tant que **tous les auteurs** n'auront pas signé une cession du droit d'auteur. Si le document n'est pas publié dans les deux années suivant ma signature des présentes, la cession sera automatiquement révoquée.

Signé à ______________________________, le ______________________
(ville et province/état) (date)

____

(signature)

[Formulaire normalisé du CNRC; aucune modification permise.]

Commentaires sur la cession du droit d'auteur
(accompagnés d'extraits de la *Loi sur le droit d'auteur*, Lois révisées du Canada 1985, chapitre C-42 modifié)

L'éditeur ne peut publier un document sans la permission ou une cession du droit d'auteur. Pour des raisons de souplesse et en vue de l'utilisation future du document publié, l'éditeur doit devenir titulaire du droit d'auteur.

Le titulaire peut céder le droit, mais cette cession doit être faite par écrit :
13.(4) *Le titulaire du droit d'auteur sur une oeuvre peut céder ce droit, en totalité ou en partie, d'une façon générale, ou avec des restrictions territoriales, pour la durée complète ou partielle de la protection; il peut également concéder, par une licence, un intérêt quelconque dans ce droit; mais la cession ou la concession n'est valable que si elle est rédigée par écrit et signée par le titulaire du droit qui en fait l'objet, ou par son agent dûment autorisé.*

Le titulaire peut être l'auteur, mais dans plusieurs cas, le titulaire est l'employeur de l'auteur:
13.(3) *Lorsque l'auteur est employé par une autre personne en vertu d'un contrat de louage de service ou d'apprentissage, et que l'oeuvre est exécutée dans l'exercice de cet emploi, l'employeur est, à moins de stipulation contraire, le premier titulaire du droit d'auteur; mais lorsque l'oeuvre est un article ou un autre contribution, à un journal, à une revue ou à un périodique du même genre, l'auteur, en l'absence de convention contraire, est réputé posséder le droit d'interdire la publication de cette oeuvre ailleurs que dans un journal, une revue ou un périodique semblable.*

La seule exception dans la cession du droit d'auteur est lorsque le document est associé au droit d'auteur de la Couronne, qui appartient au gouvernement du Canada .
12. *Sous réserve de tous les droits ou privilèges de la Couronne, le droit d'auteur sur les oeuvres préparées ou publiées par l'entremise, sous la direction ou la surveillance de Sa Majesté ou d'un ministère du gouvernement, appartient, sauf stipulation conclue avec l'auteur, à Sa Majesté et, dans ce cas, il subsiste jusqu'à la fin de la cinquantième année suivant celle de la première publication de l'oeuvre.*

Les gouvernements, en particulier le gouvernement fédéral, ne disposent pas d'un mécanisme simple de cession de droit d'auteur pour ce genre de biens de la Couronne. C'est pourquoi lorsqu'un auteur est un fonctionnaire et que, par conséquent, le droit d'auteur appartient à la Couronne, une simple permission d'usage est suffisante pour l'éditeur, pour autant que celle-ci ait été octroyée par une personne jouissant de l'autorité nécessaire pour ce faire.

Contents (concluded) / Table des matières (suite et fin)

Michael M. Mullin, Erica Goetze, Stace E. Beaulieu, and Jacqueline M. Lasker 1434–1447 Comparisons within and between years resulting in contrasting recruitment of Pacific hake (*Merluccius productus*) in the California Current System

S. Adams, B.D. Mapstone, G.R. Russ, and C.R. Davies 1448–1458 Geographic variation in the sex ratio, sex specific size, and age structure of *Plectropomus leopardus* (Serranidae) between reefs open and closed to fishing on the Great Barrier Reef

Margaret F. Merritt and Terrance J. Quinn, II 1459–1469 Using perceptions of data accuracy and empirical weighting of information: assessment of a recreational fish population

Colden V. Baxter and F. Richard Hauer 1470–1481 Geomorphology, hyporheic exchange, and selection of spawning habitat by bull trout (*Salvelinus confluentus*)

Ming Tang, Daniel Boisclair, Chantal Ménard, and John A. Downing 1482–1488 Influence of body weight, swimming characteristics, and water temperature on the cost of swimming in brook trout (*Salvelinus fontinalis*)

M.F. Leggett, O. Johannsson, R. Hesslein, D.G. Dixon, W.D. Taylor, and M.R. Servos 1489–1496 Influence of inorganic nitrogen cycling on the δ^{15}N of Lake Ontario biota

David W. Crowder and Panayiotis Diplas 1497–1507 Evaluating spatially explicit metrics of stream energy gradients using hydrodynamic model simulations

D.G. Lonzarich, M.R. Lonzarich, and M.L. Warren, Jr. 1508–1514 Effects of riffle length on the short-term movement of fishes among stream pools

Arthur J. Bulger, Bernard J. Cosby, and James R. Webb 1515–1523 Current, reconstructed past, and projected future status of brook trout (*Salvelinus fontinalis*) streams in Virginia

Jeffrey N. Houser, Stephen R. Carpenter, and Jon J. Cole 1524–1533 Food web structure and nutrient enrichment: effects on sediment phosphorus retention in whole-lake experiments

DISCUSSION DISCUSSION

Iain Suthers 1534–1536 Significance of larval condition: comment on laboratory experiments

Joel K. Elliott and William C. Leggett 1537–1538 Larval condition and vulnerability to predation: reply to comment by Suthers

National Research Council Canada
Ottawa, Canada
K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada
K1A 0R6

Postage paid at Ottawa
Publications mail
Registration No. 1240579

Port payé à Ottawa
Poste-publication
Enregistrement n° 1240579

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 57, Number 7, July 2000

Volume 57, numéro 7, juillet 2000

RAPID COMMUNICATIONS / COMMUNICATIONS RAPIDES

D.P. Swain and A.F. Sinclair **1321–1325** Pelagic fishes and the cod recruitment dilemma in the Northwest Atlantic

J.-M. Roussel, A. Haro, and R.A. Cunjak **1326–1329** Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology

ARTICLES / ARTICLES

Louise St-Cyr and Peter G.C. Campbell **1330–1341** Bioavailability of sediment-bound metals for *Vallisneria americana* Michx, a submerged aquatic plant, in the St. Lawrence River

Garry J. Scrimgeour and Patricia A. Chambers **1342–1354** Cumulative effects of pulp mill and municipal effluents on epilithic biomass and nutrient limitation in a large northern river ecosystem

Richard L. O'Driscoll, David C. Schneider, George A. Rose, and George R. Lilly **1355–1368** Potential contact statistics for measuring scale-dependent spatial pattern and association: an example of northern cod (*Gadus morhua*) and capelin (*Mallotus villosus*)

Jordan Rosenfeld **1369–1379** Effects of fish predation in erosional and depositional habitats in a temperate stream

Barry J.F. Biggs, Steven N. Francoeur, Alexander D. Huryn, Roger Young, Christopher J. Arbuckle, and Colin R. Townsend **1380–1394** Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes

Chris J. Harvey and James F. Kitchell **1395–1403** A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web

Joachim Maes and Frans Ollevier **1404–1409** Biomass transport to and from an upper estuarine area by migration of juvenile Atlantic herring *Clupea harengus*

B. Clason and G.-P. Zauke **1410–1422** Bioaccumulation of trace metals in marine and estuarine amphipods: evaluation and verification of toxicokinetic models

Antonio B.S. Poléo and Frode Bjerkely **1423–1433** Effect of unstable aluminium chemistry on Arctic char (*Salvelinus alpinus*)

Continued on inside back cover / Suite au verso

Printed in Canada by / Imprimé au Canada par
INTRA MÉDIA INC./datamark systems
Typesetting by / Typographie par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(200007)57:7;1-8